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MYCOLOGIA

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VOL. XLV MAY–JUNE, 1953 NO. 3

SCREENING TESTS DESIGNED TO DISCOVER ANTIBIOTICS SUITABLE FOR PLANT DISEASE CONTROL [1]

G. J. STESSEL, CURT LEBEN AND G. W. KEITT [2]

(WITH 2 FIGURES)

Most of the antibiotics now used in medicine have resulted from systematic searches. These materials have passed successfully a number of screening tests, many of which were devised to select antibiotics that would control bacterial or virus diseases of man and animals. Many of these materials have been tested for plant disease control and merit further work. However, in view of the remarkable accomplishments with antibiotics in medicine and of the evidence that antibiotics may be obtained in great variety and high potency, it seemed desirable to seek kinds especially adapted to plant disease research by employing screening techniques oriented toward plant pathology, rather than toward medicine. The present paper is an account of such a screening program. Especial emphasis was placed on seeking antifungal antibiotics, since most of the plant diseases for which chemicals are used at present are incited by fungi.

[1] Supported in part by the Research Committee of the Graduate School with funds supplied by the Wisconsin Alumni Research Foundation. Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Accepted for publication Nov. 2, 1952.

[2] We wish to express our appreciation to Eugene Herrling, who took the photographs.

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The work consisted of four major screening steps designed to find stable antibiotics that could be produced in quantity and that would be effective against a range of plant pathogens. The steps were (a) selection of antagonistic microorganisms from the soil, using plant pathogenic fungi as test organisms, (b) screening the selected antagonists on the basis of inhibition of a number of plant pathogens, (c) production of antibiotics by the antagonists in liquid media, and (d) studies on the stability of the antibiotics produced by selected organisms. A short account of most of this work has already appeared (**9**).

SELECTION OF ANTAGONISTIC MICROORGANISMS FROM SOIL

Primary selection tests consisted of spraying agar Petri plates containing colonies of soil microorganisms with standardized conidial suspensions of three or four fungi shown in preliminary tests to be mutually nonantagonistic. After incubating the plates, colonies that were surrounded by clear zones of inhibition of the test organisms were singled out for further study. An account of this procedure is given below.

During the autumn of 1948, 40 soil samples were collected in and near Madison, Wisconsin, and stored at 8° C until used. These samples were added to yeast extract-glucose agar [3] in dilutions previously found to give approximately 30 to 50 colonies per plate; final dilutions of soils in agar were 1 : 200,000 to 1 : 20,000,000. Approximately 20 ml of medium was used in each plate. Forty plates from each soil were incubated at 26° for 7–14 days.

After the incubation period plates were inoculated with a mixed inoculum using a spraying technique similar to those of Stansly (8) and Wilska (12). In the early part of the work each plate was sprayed for about three seconds with a standardized spore suspension containing nearly equal concentrations of conidia of *Glomerella cingulata* (Stonem.) Spauld. & v. Schrenk, *Colletotrichum circinans* (Berk.) Vogl., and *Helminthosporium victoriae* M. & M. For the major part of the work, conidia of *Verticillium albo-atrum* Reinke & Berth. were also present in the inoculum.

[3] Per l.; Bacto yeast extract, 2 gm; glucose, 10 gm; agar, 17 gm; pH after autoclaving, 6.0 to 6.4.

Conidia for spray suspensions were obtained from cultures of the fungi that had been grown on potato-glucose agar[4] at 24° for 7–14 days. The sprayed plates were incubated at 26° for two days. Soil colonies that were antagonistic to the plant pathogens were easily located at that time because of the surrounding zone of inhibition (FIG. 1). Stock cultures were made from colonies that produced large clear zones free from growth of any of the four pathogens.

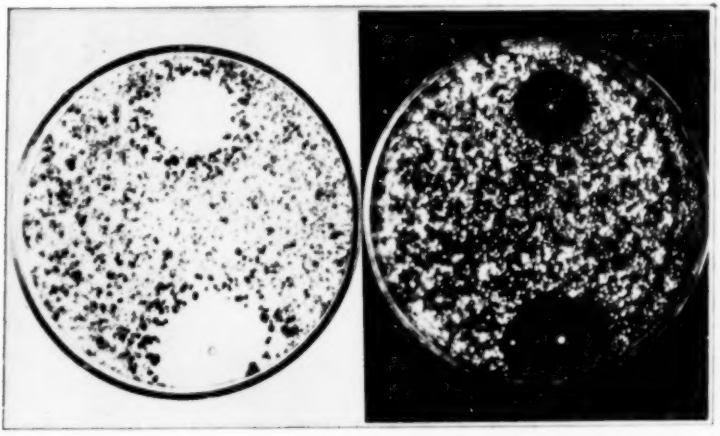

FIG. 1. A plate culture showing the inhibition of four mutually non-antagonistic plant pathogenic fungi by two organisms from soil. The picture with the light background shows growth of the two dark pathogens, *C. circinans* and *H. victoriae*, and the one with the dark background shows growth of the two light organisms, *V. alba-atrum* and *G. cingulata*.

From approximately 70,000 soil colonies that were tested by the procedure, 170 antagonistic organisms were isolated. Approximately 80 per cent of these were actinomycetes. One hundred and two of these antagonistic organisms remained for further work after the elimination of similar, mixed, or contaminated cultures.

[4] Per L.: Extract from 200 gm of sliced, peeled potatoes steamed one-half hour in 500 ml distilled water; glucose, 25 gm; agar, 17 gm; pH 6.7.

SCREENING OF ANTAGONISTS ON THE BASIS OF INHIBITION OF PLANT PATHOGENS

The second step in the screening process, the "agar streak-spray test," was a study of the relative inhibition of a number of plant pathogens by the selected antagonistic microorganisms. The test was made by streaking agar plates with an antagonistic organism, incubating the plates, spraying individual plates with one of the

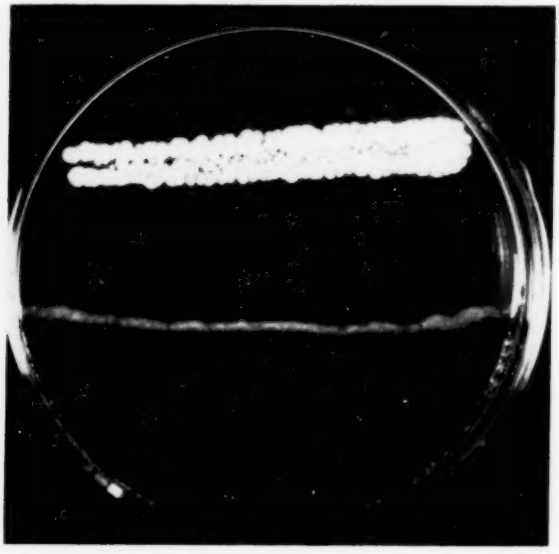

FIG. 2. The antagonism of *C. circinans* by *Streptomyces* sp. from soil.

plant pathogens, and after a further period of incubation, noting the width of the clear zone between the growth of the antagonistic organism and that of the plant pathogen (FIG. 2). The details of the test follow.

Water suspensions of antagonistic isolates were made from yeast extract-glucose agar [3] cultures grown at 26° for 7–14 days. One loopful of a given suspension was streaked on one side of a yeast extract-glucose agar plate (20 ml of medium per plate). Twenty-five or 30 plates were streaked with each antagonistic isolate. Plates were incubated at 26° for four days, and 20 plates contain-

ing the most uniform growth of each isolate were selected. Two plates of each antagonistic organism were then sprayed with a standardized suspension of one of the pathogens; in all, 10 pathogens were sprayed on the 20 plates. Sprays were prepared from cultures that had grown on potato-agar [4] at 24° for 7–14 days when fungi were used, and when bacteria were employed as test organisms, cell suspensions were derived from potato-glucose agar cultures that had been incubated 48 hours at 24°. Sprayed plates were incubated at 26° for 60–72 hours, and the distance of complete inhibition of growth of the pathogen from the antagonist was measured.

All 102 of the antagonistic isolates obtained as recorded in the first section of the paper were included in agar streak-spray tests. On the basis of the degree of inhibition of the 10 plant pathogens, 22 of the antagonists were selected for further study. These isolates were tested a second time by means of agar streak-spray tests with the 10 phytopathogens. On the basis of both tests, 21 of the isolates were selected for further studies. The plant pathogens used and examples of the degree of inhibition obtained in the second trial are given in TABLE I.

TABLE I

INHIBITION OF CERTAIN PLANT PATHOGENS BY SELECTED ANTAGONISTS IN AGAR STREAK-SPRAY TESTS

Plant pathogen	Width of clear zones produced by stated antagonist[a]		
	A67[b]	*A102*	*A158*[c]
	mm	mm	mm
Agrobacterium tumefaciens (Smith & Towns.) Conn.	35	15	0
Colletotrichum circinans (Berk.) Vogl.	65	38	29
C. pisi Pat.	30	25	25
Erwinia amylovora (Burr.) Winslow *et al.*	57	12	20
Fusarium oxysporum f. *lycopersici* (Sacc.) S. & H.	15	19	20
F. oxysporum f. *pisi* (Linford) S. & H.	17	18	20
Glomerella cingulata (Stonem.) Spauld. & v. Schrenk	24	24	25
Helminthosporium victoriae M. & M.	65	28	27
Sclerotinia fructicola (Wint.) Rehm	18	20	30
Verticillium albo-atrum Reinke & Berth.	22	31	30

[a] Each entry is the average from two plates.
[b] Produces toximycin (11).
[c] Produces helixin (6).

THE PRODUCTION OF ANTIBIOTICS BY ANTAGONISTS IN LIQUID MEDIA

The purpose of the third step in the screening procedure was to determine which of the 21 selected antagonistic isolates could produce antibiotics in shaken liquid media, since liquid cultures were necessary for obtaining sufficient amounts of antibiotics for fractionation and further study.

To determine whether an antibiotic was present in the liquid media in which the microorganisms had grown, the Petri plate assay method of Dunshee *et al.* (1), employing the fungus, *G. cingulata,* was used with certain modifications. Blotting paper discs were replaced by "penicylinders." The latter were placed on a 10 ml base layer of agar and sealed in place with 5 ml of agar seeded with the fungus. Each sample was tested by filling six cylinders, three in each of two plates. Plates were incubated at 26° for 24–35 hours and the size of inhibition zones noted.

Five hundred ml Erlenmeyer flasks containing 100 ml of liquid medium per flask were used to culture the antagonistic isolates. These were divided into three groups of seven isolates each, and each group was tested at a different time. Flasks were shaken for four days at 26° on a reciprocating shaker making 65 cycles per min. with a 15 cm stroke. The media used in the experiments are given in TABLE II; three replicate flasks were used in each antagonist-medium combination. Suspensions for seeding the flasks were made from cultures of the antagonists that had grown on yeast extract-glucose agar [3] in bottle slants at 26°. Cultures of actinomycetes were 7–14 days old; cultures of isolate *A67* (a bacterium) were 48 hours old. Inoculation was at the rate of 5 ml of spore or cell suspension per flask. After the fermentation period, samples were prepared for assay by removing the cells and sediment either by filtration, using pipette, containing a cotton-filled base, or by centrifugation.

It was found that 16 of the 21 organisms produced diffusible antibiotic materials, as indicated by the production of inhibition zones, when grown in one or more media. The 12 isolates producing zones of complete inhibition of 17 mm or more in diameter were used in a second fermentation test; the medium employed for

TABLE II

MEDIA USED IN TESTS FOR PRODUCTION OF ANTIBIOTICS BY ANTAGONISTS[a]

Ingredient	Gm per liter of ingredient in stated medium:				
	A	B	C	D	F
Beef extract	—	—	—	—	3
$CaCO_3$	—	1.5	5	1.5	—
Corn steep[b]	—	50	10	—	—
Glucose	10	10	20	20	10
Peptone	—	—	—	—	10
Soybean meal	—	—	20	40	—
Yeast extract	2	—	—	—	—

[a] Usual pH of media after autoclaving: A, 5.2; D, 7.2; and F, 6.2. Media containing corn steep (B, C) adjusted to pH 7 before adding $CaCO_3$ and autoclaving. Final pH of these media usually 7.1–7.2.
[b] Solids, 50–60 per cent.

each organism was that most favorable for antibiotic production in the first test. On the basis of the results of both of these tests, as well as the results of the agar streak-spray tests, 8 of the 12 isolates were selected for further study.

STABILITIES OF ANTIBIOTICS PRODUCED BY ANTAGONISTS

The property of an antibiotic to retain its potency under different conditions may determine the methods by which a crude material is purified and its ultimate usefulness in plant disease studies. Culture centrifugates of the eight antagonists previously selected were subjected to a series of tests designed to give some assessment of the stability of the antibiotics produced by these organisms. In part the procedures suggested by Johnson *et al.* (2) were used.

The stability of the antibiotics in culture centrifugates at different pH levels was studied by adjusting the centrifugates to pH 6 and removing five ml. aliquots, which in turn were adjusted to five pH levels from 2 to 10, inclusive. Samples were held at the variout levels for four hours at 29–30°, readjusted to pH 6, made to 10 ml., and then assayed. A dosage-response curve was constructed for the antibiotic produced by each isolate on the basis of inhibition by four two-fold dilutions of the original centrifugate at pH 6; activity of treated samples was determined by interpolation of this curve.

On the basis of the assay results a curve was plotted for each culture centrifugate, using as coordinates the treatment pH and the percentage of the activity remaining after treatment. From this curve the critical pH range (the range over which at least 50 per cent activity was retained) and the optimum pH (the pH for greatest stability) were determined.

To test heat stability, aliquots of culture centrifugates at the optimum pH value were held at 100° for periods of from three minutes to 20 hours; other aliquots were autoclaved. Activity of treated samples was determined by means of dosage-response curves based on activity of different dilutions of untreated samples.

Time stability studies were made on centrifugates that were adjusted to the optimum pH value and stored at 8°, 26°, or 36° after the addition of a small amount of toluene as a preservative. Samples were assayed at intervals up to the time the sample had lost 50 per cent of its original activity or until the experiment was concluded, at 60 days. Decrease in activity of samples at 26° and 36° was calculated at each sampling time on the basis of a dosage-response curve produced by the sample kept at 8°. At each sampling time the approximate decrease in activity of the 8° sample was estimated by reference to the dosage-response curve that had been prepared for this sample at the start of the experiment. Suitable corrections were made in the calculation of the loss in activity of samples at 26° and 36°.

On the basis of the three types of stability tests it was concluded that six of the eight antagonists produced antibiotics that were of sufficient interest for further study. Two of these materials seemed to have special merit; these were later named helixin and toximycin. Of the two antagonists that were not selected for further study, one, a *Streptomyces* species, was eliminated because it was identical with or similar to the *Streptomyces* sp. that produces antimycin (**4**), since the morphology of the organisms, their growth habits on a number of media, and the properties of the antibiotics produced by them were remarkably similar. No attempts were made to isolate antimycin A from cultures of the organism obtained in the present work.

The six organisms that passed the screening tests were studied determinatively; it was shown that five belonged to the genus *Streptomyces* Waksman & Henrici, and that the sixth was *Bacillus subtilis* Cohn *emend.* Prazmowski. The morphological, cultural, and physiological characteristics of four of the *Streptomyces* species and of *B. subtilis* (our isolate *A67*) are given in detail elsewhere (**10**). The fifth *Streptomyces* isolate and the antibiotics it produces, the helixins, have been described in other papers (**6, 7**).

DISCUSSION

Isolation and screening procedures were designed and employed to search for antibiotics that could be used in plant disease control work. It seemed reasonable to expect that materials selected by these methods would be sufficiently stable for ordinary fractionation procedures and for tests with plant diseases. Because four plant pathogenic fungi were used in the primary isolation work and fungi were used in the second selection step, it was hoped also that the antibiotics that passed all of the screening tests would have activity against a number of fungi. Other work (**3, 5, 6, 7, 11**) has shown that two of the selected antibiotics that have been given further study are active against many fungi, possess reasonable stability, and have potentialities for plant disease control work. It would seem, therefore, that systematic studies of the type described in this paper should be of value in discovering antibiotics that may be of interest to the plant pathologist. These materials may also merit investigation in other fields, since they may act on groups of organisms (*e.g.*, the fungi) rather than individual species or lines.

SUMMARY

Screening methods aimed at finding organisms that produce stable antibiotics which inhibit a range of phytopathogens are described. Agar plates, each containing colonies of 30–50 soil microorganisms, were sprayed with four mutually nonantagonistic phytopathogenic fungi. After a period of incubation, organisms that were antagonistic to the four phytopathogens, as indicated by zones of inhibition, were isolated. The degree of antagonism exerted by

these organisms on 10 phytopathogens was assessed in tests in which individual phytopathogens were sprayed on agar plates that had been streaked four days previously with a single antagonistic organism. The antagonists that were the most inhibitory were grown in five liquid media. The stability of the antibiotics in culture centrifugates was assessed with respect to pH, heat, and storage. Out of approximately 70,000 colonies of soil microorganisms, an isolate of *Bacillus subtilis* and five *Streptomyces* isolates were selected for further work.

DEPARTMENT OF PLANT PATHOLOGY,
UNIVERSITY OF WISCONSIN,
MADISON, WISCONSIN

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THE INFLUENCE OF NITROGENOUS COMPOUNDS ON THE GROWTH OF HELMINTHOSPORIUM GRAMINEUM IN CULTURE[1]

RICHARD H. CONVERSE[2]

(WITH 4 FIGURES)

Helminthosporium gramineum Rabh., the causal organism of the stripe disease of barley, exhibits a high level of parasitism. The fungus resembles the seedling-infecting smuts in many ways. It attacks only young seedlings, invading their shoots systemically and evoking severe symptoms in the host leaves as they mature. The existence of physiologic races of the pathogen has been reported by several workers (**1**, **4**, **9**, **10**). Barley varieties differ in their levels of resistance to this disease (**8**, **11**).

Because *H. gramineum* possesses many of the attributes of a specialized facultative saprophyte yet grows readily on synthetic culture media, it was used for a series of investigations on the effect of barley plant products and of nitrogenous compounds on mycelial growth.

Preliminary experiments indicated that the addition of expressed barley plant sap to a glucose salts basal medium quadrupled the growth of the fungus. There were small differences between varieties in this respect, but no consistent trend was found. Substances diffusing from barley germinating on a glucose salts agar increased average colony diameters of the fungus 6–8% as compared to comparable controls. These differences were small but

[1] A portion of a thesis presented to the Faculty of the Graduate School of the University of California January, 1951 in partial fulfillment of the requirements for the degree Doctor of Philosophy. The author acknowledges the aid and advice of several members of the Division of Plant Pathology of the University of California and particularly the help of Dr. B. R. Houston under whose guidance this work was done.

[2] Present address, Oklahoma Agricultural Experiment Station, Stillwater, Okla.

highly significant statistically when an analysis of variance was performed, and paralleled the differences in stripe disease susceptibility of the two barley varieties tested.

The studies to be reported were designed to attempt duplication in chemically defined media of the stimulation observed by these barley products.

MATERIALS AND METHODS

Single spore isolates or lines of *H. gramineum* of known history were used throughout this study. Isolates A and B were recovered from naturally infected barley in California, while isolate C, a stable strain of the fungus found to be pathogenic to two susceptible California barley varieties, was obtained from the University of Wisconsin. Isolate A was used during the first part of these investigations. It was found to sector so frequently that isolate C was substituted for later nutrient studies. Where comparisons between two isolates were desired, isolate B was used.

Stock cultures were maintained on potato glucose agar. The primary inoculum for all nutrient studies consisted of small uniform agar discs from advancing margins of week-old stock cultures grown in petri dishes.

The following basic medium, found by Houston [3] to be the best carbon-salts combination for growth of this fungus, was used for all experiments:

Glucose	40 g.
Nitrogen source to furnish	0.245 g.
KH_2PO_4	0.30 g.
K_2HPO_4	1.20 g.
$MgSO_4 \cdot 7H_2O$	0.30 g.
$FeCl_3 \cdot 6H_2O$	1 ppm
$ZnSO_4 \cdot 7H_2O$	1 ppm
$CuSO_4 \cdot 5H_2O$	1 ppm
$MnSO_4 \cdot 5H_2O$	1 ppm
Distilled water	1000 ml.

This medium, using an amount of NH_4NO_3 (0.70 g./l.) known to furnish twice the optimum nitrogen requirement for the fungus, was used as the control and will hereafter be referred to as the basal medium.

[3] Unpublished information from Dr. B. R. Houston, Division of Plant Pathology, University of California, Davis, California.

When growth was to be measured in nutrient solutions, these solutions were portioned in 50 ml. amounts into 250 ml. Erlenmeyer flasks.

Each replicate was handled identically. An inoculum disc of standard diameter was aseptically placed on the bent end of a glass rod which passed through the cotton plug of the Erlenmeyer flask containing the nutrient solution. The disc was allowed to touch the solution. The flask was placed in the dark at 25 ± 1° C for the required period with a minimum of disturbance. The resulting compact mycelial mat was washed repeatedly, air dried to a constant weight, and weighed. The average dry weight of mycelium was taken as the measure of growth for all media. Average values were usually based on five replicates. In general variation between replicates of a treatment in a given experiment was moderate, while differences between isolates and within a given isolate over time were often quite marked.

The pH of the residual nutrient solution was determined by means of a Beckman pH meter, Model G. If the culture medium remaining was to be analyzed for nitrate or ammonium content, it was reautoclaved and stored under refrigeration until used. Nitrate was determined colorimetrically by the technique of Blom and Treschow (**3**) as modified by the late Dr. W. O. Williams of the University of California. Ammonia determinations were made according to the A.O.A.C. method 12.58 (**2**) as modified by the Division of Agronomy, University of California.[4]

EXPERIMENTAL RESULTS

Inorganic nitrogen preferences. Ammonium nitrate was added to the basal medium as the nitrogen source for *H. gramineum*. The rate of preferential utilization of ammonia could be followed by noting the progressive pH changes of the medium. The ammonium and nitrate concentrations and observed pH values of media incubated with isolate C for various lengths of time are presented in FIG. 1. The pH-time curve follows the drop-and-rise

[4] The writer is indebted to Mr. Douglas Stuart of the Division of Agronomy, University of California, Davis, California for assistance in making the ammonia determinations.

pattern expected for a fungus that utilizes ammonia in preference to nitrate during the early period of growth. A theoretical pH for each lot of medium was calculated from the concentration of ammonia and nitrate left in the medium by each mycelial pad at a given time and a knowledge of the buffer capacity of the medium.

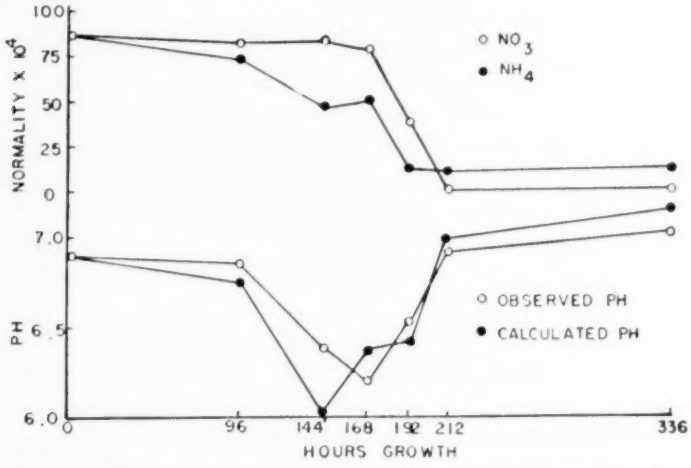

FIG. 1. Changes in average ammonia and nitrate concentrations and observed and theoretical pH values of media residues of individual cultures when incubated with isolate C of *H. graminicum* at intervals up to 336 hours at 25° C.

These theoretical pH values, plotted in FIG. 1, follow the general pattern of the pH values originally determined for the solutions. Comparable curves for isolate B are similar. By the "t" test for paired items the fit between observed and calculated pH values in FIG. 1 is poor and would occur only one time in one hundred by chance. However, errors introduced by autoclaving spent media may account for much of this disparity, and the changes in nitrate and ammonium nitrogen calculated for spent media are assumed to represent utilization trends of the fungus. Only 10% of available nitrate was used during the first 150 hours of growth, while 50% of the ammonia was taken up from the medium in the case of both isolates. At 225 hours nitrate removal became complete for both isolates, while ammonia removal approached 100% asymptotically.

The pH-time curves of isolates B and C resembled each other. Similar curves for isolate A were of the same general shape except that the magnitude of the pH drop and the rate of subsequent rise were much greater, values as low as pH 3.5 being obtained at 168 hours, followed by a change to pH 6.4 in 24 hours. Determination of nitrate and ammonia levels in media incubated with isolate A were not made because of its instability in culture.

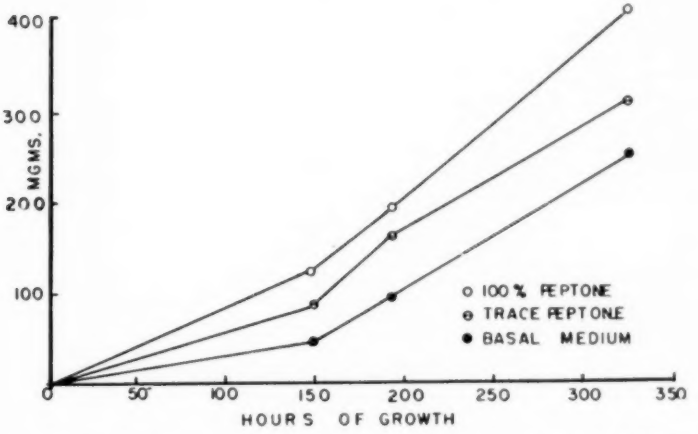

FIG. 2. Average dry mycelial weights of isolate A grown in peptone medium, trace peptone medium, and basal medium at 25° C over a series of time intervals.

Amino acid preferences. The response of the fungus to the addition of peptone to the basal medium is shown in FIG. 2. When Difco Bacto Peptone was used to furnish 100% or 5% of the total nitrogen of the medium, marked increases of growth of isolate A occurred as compared to that in basal medium. Similar but less marked stimulation occurred when isolate C was used.

The vitamins biotin, riboflavin, thiamin, pantothenic acid, inositol, nicotinic acid and pyridoxine, known to be present in the peptone, were added singly and in combination to the basal medium. Pyridoxine was supplied at the rate of 125 mmg./l. and nicotinic acid at 100 mmg./l.; all other vitamins at 10 mmg./l. In all instances these vitamins failed to increase the growth of isolate C over that

obtained in basal medium alone. Elliott (5) also failed to find vitamins of the B complex of value to this fungus.

Amino acids were used singly as nitrogen sources. In each case the amino acids furnished half of the nitrogen supply of the medium, the rest being supplied by ammonium nitrate. The ammonium nitrate present in the amino acid media represented a near-optimum supply of inorganic nitrogen for this fungus. Therefore any stimulatory or depressing effect of the added organic

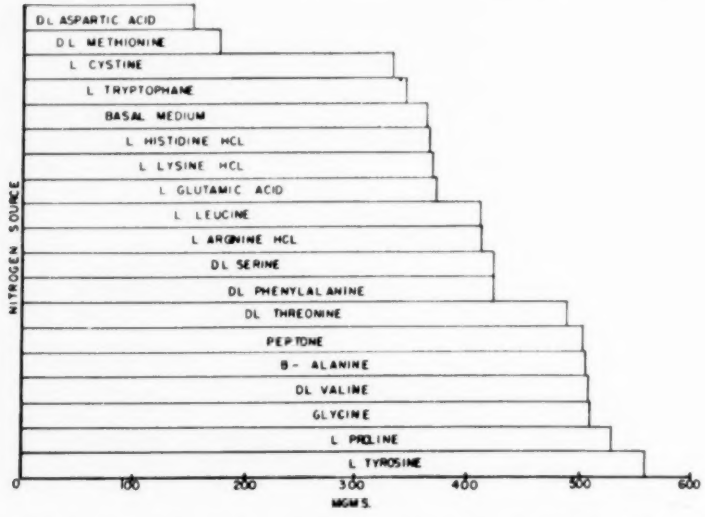

FIG. 3. Mycelial weight responses of isolate C of *Helminthosporium gramineum* to various amino acid media after 336 hours growth at 25° C.

nitrogen could be compared directly with the effect of the excess supply of inorganic nitrogen in the basal medium. FIG. 3 presents the average mycelial weights of isolate C at 336 hours obtained using various amino acids as compared to peptone and to ammonium nitrate as nitrogen sources.

Six distinct mycelial weight groups were obtained with these amino acid media when the data were subjected to an analysis of variance. The media falling in a given weight group produced statistically homogeneous growth responses. There were statisti-

cally significant differences between weight groups. The weight groups were: I, DL aspartic acid and DL methionine; II, L cystine and L tryptophane; III, basal medium, L histidine HCl, L lysine HCl, and D glutamic acid; IV, L leucine, L arginine HCl, DL serine, DL phenylalanine; V, LD threonine, peptone, β-alanine, DL valine, glycine; and VI, L proline and L tyrosine. It will be noted that six of the amino acids supported a growth as great or

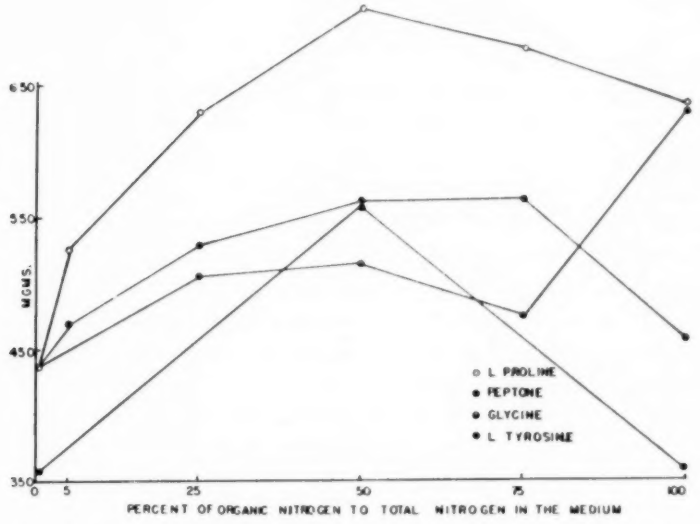

FIG. 4. The effect of changing the per cent of amino acid nitrogen supplied in media, total nitrogen being constant for each medium, on the average dry weight of mycelium of isolate C in milligrams after 336 hours of growth at 25° C.

greater than that supported by peptone. The stimulation by these amino acids and by peptone approached the growth response evoked by barley plant extracts.

Not only did amino acids differ greatly among themselves as to their ability to stimulate the growth of the barley stripe fungus, but the proportion of total nitrogen furnished by a given amino acid had marked influence on its stimulatory effect. In FIG. 4 the effect on growth of isolate C of increasing the per cent of organic-nitrogen in the medium while keeping total nitrogen constant is

illustrated. With the exception of the aberrant response of the 100% glycine nitrogen medium, peptone and the amino acids proline and glycine were most stimulatory when they supplied half the medium nitrogen. The tyrosine curve of FIG. 4 follows this same pattern. However, the data for this amino acid were obtained separately from those for the other materials and are not directly comparable, as the weights of mycelium formed in the basal medium controls were abnormally high in this latter experiment.

DISCUSSION

The barley stripe disease fungus was found to make some growth on all of the nitrogen sources tested. None of the B vitamins used stimulated growth over comparable basal medium checks. The fungus belongs among the *Helminthosporium* species reported by Lilly and Barnett (**7**) as able to utilize nitrate nitrogen. Ammonia and amino nitrogen were also utilized. However, the growth promoting abiiity and preferential use of all these nitrogen sources were not the same. No attempt will be made to explain the physiological significance of the mycelial weight groups found when comparing amino acids.

If the quantitative amount of mycelial growth supported by a given nutrient is assumed to reflect its nutritional value to the fungus and to the survival of the fungus in nature, then certain amino acids are of much greater nutritional value than inorganic nitrogen or other amino acids. For example, L tyrosine stimulated growth (of isolate C) increasing mycelial weight over controls up to 150%. Using other isolates (isolates A and B, respectively) the author found barley extracts to give 400% of control weights and products diffusing from germinating barley to give 108% of control weights.

Hrushovetz (**6**) has stated that *Helminthosporium sativum* root rot affects the concentration of amino acids in wheat roots. The pathogenicity of *H. sativum* is influenced by the kinds of amino acids supplied to the inoculum in culture. Wain and Wilkinson (**12**) found that organic solutes including amino acids diffused from germinating pea seeds into the environment. The relation of the nutrition and parasitism of fungi is a highly speculative field.

It is possible that the amino acid status of barley seedlings may exert some influence on the susceptibility of the seedlings to the stripe disease. Because no studies were undertaken of the amino acid status of seedlings of the various barley varieties used, the data here reported are admittedly inconclusive as to the role played by the amino acid status of the host in determining the inception of the barley stripe disease. The data do indicate a possible relationship.

SUMMARY

Helminthosporium gramineum Rabh. exhibited a marked growth response in culture to barley plant extracts and to products diffusing from germinating barley. The organism utilized the ammonium ion in preference to nitrate ion in early stages of growth. Peptone was found to stimulate growth. The B vitamins present in peptone were not found to increase fungus growth when added to basal medium. Growth in certain amino acids plus ammonium nitrate was much greater than that in ammonium nitrate as the sole nitrogen source. In the presence of L tyrosine, L proline, glycine, DL valine, β-alanine, or DL threonine, fungus growth equalled or exceeded that in peptone and approached that obtained with barley plant extracts. Amino acids were the only nutrients tested that gave this type of response. The possibility of a relationship between the response of *H. gramineum* to amino acids in culture and the establishment of the fungus in the host is suggested.

DIVISION OF PLANT PATHOLOGY,
UNIVERSITY OF CALIFORNIA,
DAVIS, CALIFORNIA

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OBSERVATIONS ON STREPTOMYCES GRISEUS. V. SYNTHETIC MEDIA FOR VITAMIN B_{12} PRODUCTION WITH SPECIAL REFERENCE TO POSSIBLE VITAMIN B_{12} PRECURSORS

EUGENE L. DULANEY AND PATRIC L. WILLIAMS

Since the original isolation of vitamin B_{12} from liver (**19**), a number of papers have reported the isolation of the same vitamin from microbial metabolism solutions, thus indicating a practical source of the compound (**13**, **14**, **15**, **16**, **18**, **23**). This capacity for vitamin B_{12} production appears to be widespread in the genus *Streptomyces*, including the antibiotic-producing species.

One of the primary problems that must be overcome in putting a new microbial product into production is that of devising a suitable medium. Such media are usually composed of complex organic materials that have the two advantages of being inexpensive and of supporting high-broth potencies of the compound in question. These complex organic media, however, are frequently not useful as tools in the study of various problems that arise, and it becomes necessary to develop simple synthetic media to aid in the elucidation of these problems. The present paper treats of the development of some simple synthetic media for use in searching for possible vitamin B_{12} precursors and in studying the relationship between growth and vitamin B_{12} production.

METHODS

The basal medium used in these studies was a modification of the glucose-inorganic salts medium used previously in our studies on synthetic media for streptomycin production (**8**, **9**). Preliminary experiments indicated that maximum yields of vitamin B_{12} were obtained when the glucose and $(NH_4)_2HPO_4$ levels were raised and Co^{++} as $Co(NO_3)_2 \cdot 6H_2O$ was added. The composition

finally decided on was glucose 20.0 gm, $(NH_4)_2HPO_4$ 7.5 gm, NaCl 5.0 gm, K_2HPO_4 2.0 gm, $MgSO_4 \cdot 7H_2O$ 1.0 gm, $CaCl_2$ 0.4 gm, $FeSO_4 \cdot 7H_2O$ 10.0 mg, $ZnSO_4 \cdot 7H_2O$ 10.0 mg, $Co(NO_3)_2 \cdot 6H_2O$ 10.0 mg, and distilled water to one liter. This level of Co^{++}, 2γ/ml, is in excess of the amount required for maximum vitamin B_{12} production but is well below the inhibitory level.

This medium is prepared by dissolving the salts in two-thirds of the water and the glucose in one-third of the water and combining them aseptically after autoclaving at 15 lbs pressure for 16 minutes. When possible the compounds being tested as precursors were added aseptically to the medium after sterilization in one ml. amounts so that the final desired concentration was obtained. The pH of the medium, even after supplementation with the adjuvants, was never below 6.5 nor above 7.3.

The strain of *Streptomyces griseus* used in these experiments is a mutant that has descended from the strain used previously in our studies on streptomycin production (**8**, **9**) and is strain G of paper IV of these series (**10**). Spores of this strain, lyophilyzed in sterile soil, served as the stock from which all cultures used in these experiments descended. Spores in soil were spread over slants of a nutrient agar medium with a soybean meal base. After incubation at 28° C for seven days, excellent spore crops had developed which were harvested by washing with sterile distilled water. One ml of this spore suspension was used to start each flask of vegetative inoculum. The vegetative inoculum was always developed in the glucose-salts medium, 30 ml/125 ml flask, on rotary shakers moving at 220 rpm and describing a circle one inch in diameter. After incubation for three or four days at 28° C, one ml of the vegetative growth that had developed was used to inoculate each fermentation flask. The fermentations, which were incubated under the same conditions as was the inoculum, were sampled at appropriate intervals, diluted in M/20 phosphate buffer, autoclaved at 15 lbs pressure for 15 minutes, and assayed by the cylinder plate method using Davis' *Escherichia coli* mutant as the test organism (**7**). The Dorner strain of *Lactobacillus lactis* was used as the test organism, however, when the medium contained methionine.

RESULTS AND DISCUSSION

Nitrogen sources for growth and vitamin B_{12} production. The [illegible] salts medium supports vitamin B_{12} broth potencies of approximately 3000 u./ml thus showing that *Streptomyces griseus* can synthesize the complex B_{12} molecule from glucose carbon and inorganic salts.

It appeared possible that increased yields could be obtained by the substitution of other carbon and nitrogen sources for the glucose and the $(NH_4)_2HPO_4$. Thus, some twenty-two nitrogen sources, principally single amino acids, were substituted for the $(NH_4)_2HPO_4$ at a level of 3.732 mg N/ml. The compounds tested and the maximum results obtained are given in TABLE I.

TABLE I

NITROGEN SOURCES FOR GROWTH AND VITAMIN B_{12} PRODUCTION

Nitrogen source	Maximum vitamin B_{12} broth potency	Day of maximum
$(NH_4)_2HPO_4$	3,100	5
N-Z-amine	6,700	4
Urea	1,500	4
DL-α-Alanine	6,000	4
β-Alanine	8,300	7
L(+)Arginine HCl	1,000	7
Asparagine	5,400	4
DL-Aspartic acid	4,600	7
L(+)Cysteine HCl	3,000	7
L(-)Cystine	1,000	6
Creatine hydrate	no growth	
Creatinine	no growth	
L-Glutamic acid	3,000	6
Glycine	6,100	6
L-Histidine HCl	5,400	4
DL-Isoleucine	no growth	
L(-)Leucine	1,600	7
DL-Lysine HCl	1,400	7
DL-Methionine	700	6
L(-)Proline	15,000	6
DL-Serine	inhibition	
DL-Threonine	5,600	7
DL-Valine	no growth	

With one exception, the single amino acids, namely DL-alpha-alanine, Beta-alanine, glycine, L-histidine HCl, L(-)proline and L-arginine HCl, that had been found previously (8) to support relatively high streptomycin yields, also supported relatively good vitamin B_{12} yields. The one exception was arginine, and in this instance growth was slow and good cell weight were not obtained.

Had the experiment been prolonged past seven days, as was done in the previous test for streptomycin production, perhaps higher vitamin B_{12} broth potencies would have been realized. Two other amino acids, namely L-aspartic acid and DL-threonine, that had supported little if any streptomycin production, supported relatively good yields of vitamin B_{12}. DL-lysine HCl and DL-methionine supported somewhat lower yields of vitamin B_{12} and had been found previously to support little or no streptomycin production. Creatine, creatinine, DL-valine, and DL-isoleucine when used as single nitrogen sources, did not support growth.

There is a much closer correlation between growth and vitamin B_{12} production than there is between growth and streptomycin production. In all treatments where growth occurred, with one exception, some vitamin B_{12} was produced. The one possible exception was in the medium containing DL-serine. These metabolism solutions were inhibitory to the test organism, *Lactobacilus lactis*. It is still possible that these broths contained vitamin B_{12}. There is also an apparent correlation between the amount of growth obtained and the vitamin B_{12} level produced—a point that will be dealt with more fully later. The highest B_{12} broth potencies and the highest cell weights were obtained in the medium containing L(-)proline as the sole nitrogen source. Proline, as a single nitrogen source, had been found previously to support quite high streptomycin activities (**8**). It would appear that the series of mutant strains with which we have been working might well be termed "proline specific." The use of proline as a single nitrogen source has a marked effect on the physiology of these strains but not necessarily on other strains.

Carbon sources for growth and vitamin B_{12} production. The carbon sources used as substitutes for glucose were limited, with the exception of sucrose, to those that had been found previously (**9**) to support good growth of *Streptomyces griseus*. The possible substitutes were tested at a level of two per cent. The results of this test are given in TABLE II. None of these substitutes was particularly outstanding, although lactose, maltose, dextrin, starch, and mannitol supported yields that were significantly better than those obtained by the use of glucose. Perhaps in experiments

such as these, marked increases in broth potencies should not be expected. Practically nothing is known of the factors that limit vitamin B_{12} synthesis, and the mere substitution of single carbon and nitrogen sources may not be satisfying these limitations. Whatever these limitations are, later results have made it clear that the levels of carbon and nitrogen used in these experiments were above the optimum required for maximum growth and vitamin B_{12} production.

TABLE II

CARBON SOURCES FOR GROWTH AND VITAMIN B_{12} PRODUCTION

Carbon source	Maximum vitamin B_{12} broth potency	Day of maximum
Cellobiose	2600	3
Dextrin	4500	3
Galactose	3200	3
Glucose	3100	3
Glycerol	2400	3
Lactose	4500	6
Levulose	3100	6
Maltose	4700	6
Mannitol	3800	3
Mannose	3100	6
Starch	4200	3
Sucrose	900	3
l-Xylose	3400	6

Precursor studies. In a search for biological precursors, one is faced with a number of problems of which the primary one is that of the criteria to be used in judging as to whether or not a compound can serve as a precursor. The ultimate test for precursor activity would appear to be the use of "tagged" atoms in proving that the compound under test was assimilated into the molecule in question. This is impractical on a large scale, and we are faced with the necessity of using other methods. In these experiments, the preliminary basis for judging precursor activity was whether or not a compound, when added to a medium being metabolized by growing cells, resulted in an apparent increase in vitamin B_{12} broth potency. The weakness of this criterion is fully realized, and this point will be expanded somewhat later. It is within the bounds of this convention, however, that we speak of compounds that stimulate B_{12} production when an apparent increase in B_{12} broth potency is found in treatments to which these compounds are

added. Of course one expects an increase upon addition of a single compound only if the limiting reaction is being fed.

At the outset of our search for B_{12} precursors, very little was known about the structure of the vitamin B_{12} molecule. The presence of cobalt was known, and this was incorporated into our basic medium (**16**, **20**). Under such conditions, our initial search for precursors became quite empirical. It involved the addition of complex organic plant and animal remains to the glucose-$(NH_4)_2HPO_4$-salts medium in the hope that a marked stimulation of broth potency would occur. This would then be followed by fractionation of the substance invoking the stimulation. The list of materials tested is long and need not be detailed but included fish meals, seed meals, yeast products, casein hydrolysates, distillery residues, etc. All of these materials were tested at a level of 5 mg/ml. In no instance was there an increase in broth potency that could not be attributed to an increase in cell weight.

Following this unsuccessful test of the above materials, a number of biologically active compounds, namely amino acids, organic acids, purines, and pyrimidines, were screened as possible precursors. All of the amino acids listed in TABLE I were added singly at a level of 0.46 mg/N/ml. The carboxylic acids, *i.e.*, fumarate, malate, pyruvate, acetate, citrate, lactate, succinate, and ethloxalacetate were used at a level of 5 mg/ml, whereas adenine, guanine, xanthine, uracil, orotic acid, cytosine, thymine, and yeast nucleic acid were tested at a level of 1 mg/ml. Detailed results of these experiments need not be given, but it will suffice to report that the use of none of these compounds resulted in precursor-like activity. In several instances, significant increases in broth potencies were obtained. Here again, however, these increases were not marked and could be attributed directly to stimulation of growth.

All of the above materials were tested at a time when little was known about the structure of the B_{12} molecule. In retrospect, most of the materials should not have been expected to show precursor activity, but some of them would have been selected for testing on the basis of their structures.

With increase in knowledge of the structure of the B_{12} molecule, we are able to test for precursor activity compounds whose formulations occur in the molecule. Here we are on much firmer ground in our search for precursors.

At the present time the vitamin B_{12} molecule is known to contain cobalt (**20**), 1-amino-2-propanol (**25**), α-ribazole phosphate (1-α-D-ribofuranosido-5,6-dimethyl benzimidazole phosphate) (**2, 3, 4**), and an unknown portion which has been suggested as being a pyrrole or porphyrin-like (**1**).

All of the portions of the B_{12} molecule, *i.e.*, amino propanol, α-ribazole and the unknown moiety, have been tested singly, at a level of 100 γ/ml and in all possible combinations. In no instance was any increase in B_{12} broth potency noted. The failure of amino-propanol to stimulate B_{12} production was followed by a test of L-threonine and hydroxyglutamic acid. Amino-propanol could be formed by the decarboxylation of threonine and hydroxyglutamic acid could be decarboxylation go to threonine and thence to amino-propanol. The L-threonine and hydroxyglutamic acid, as well as N-acetyl hydroxyglutamic acid, were tested singly at levels of 100 γ/ml and in combination with the other parts of the molecule. The results did not indicate any stimulation of B_{12} production.

The compounds tested as possible precursors for the unknown part of the molecule were selected on the basis that this unknown moiety is a pyrrole or porphyrin-like. The unknown moiety, singly at a level of 100 γ/ml or in combination with α-ribazole and/or amino-propanol, did not increase the B_{12} broth potency. Pyrrole and L(-)proline likewise elicited no response when tested singly or with the other fragments of the molecule. Bilirubin, chlorophyll, cobalt protoporphyrin or cobalt hematoporphyrin, singly at a level of 100 γ/ml or in combination with α-ribazole and/or amino-propanol were inactive as precursors. It should also be noted that glycine, which has been shown to be a precursor of porphyrins (**22**), was also inactive in increasing B_{12} broth level.

Following our failure to find precursors for the amino-propanol and unknown part of the molecule, our attention was turned to the α-ribazole phosphate moiety. The α-ribazole singly, at several

concentrations up to 100 γ/ml or in combination with amino propanol or with the unknown fraction did not induce a rise in the level of B_{12} produced. This was likewise true of 5,6-dimenthylbenzimidazole and 2,3-diamino-5,6-dimethyl benzene. As was expected 2,3-diamino-5,6-dichlorobenzene was quite inhibitory. o-Phenylenediamine (diamino benzene), when used singly, induced a marked increase in the apparent level of B_{12} in the broth. Likewise o-nitroaniline, o-xylidine, and 3,4-diaminotoluene, when used singly, stimulated B_{12} production. However, the use of benzene, aniline, or nitrobenzene did not result in a stimulation.

p-Aminobenzoic acid, reported by Davis (**6**) to be involved in B_{12} synthesis by *Escherichia coli*, was inactive in stimulating B_{12} production by *Streptomyces griseus*. This was likewise true of benzoic acid, quinic acid, and shikimic acid, the last of which has been reported to be an important intermediate in the synthesis of cyclic compounds (**5**). Other benzene derivatives, *i.e.*, sodium hydroxybenzoate, saccharine, p-dimethylaminobenzaldehyde, benzlycarbinol, and sulfanilic acid, were inactive in stimulating B_{12} production.

In summary, of all of the compounds tested, only o-phenylenediamine, o-nitroaniline, o-xylidine, and 3,4-diaminotoluene have invoked a marked increase in B_{12} broth potency. The relationship between the structure of these compounds and the B_{12} molecule is obvious. However, α-ribazole, 5,6-dimethylbenzimidazole, and 2,3-diamino-5,6-dimethylbenzene are structurally closer to the B_{12} molecule than are any of the stimulatory compounds. Moreover, these three compounds show vitamin B_{12} activity in the rat (**11**, **12**). Wooley has presented evidence (**26**, **27**) that the diaminodimethylbenzene increased both the B_{12} and B_2 production by *Bacillus megatherium*. This prompted us to test a series of possible riboflavin inhibitors in the hope that a shift to B_{12} synthesis would follow. These compounds, tested at a level of 25 γ/ml, included 2-desoxy-2-imino-riboflavin, isoriboflavin, phosphorylated isoriboflavin, galactoflavin, 6,7-dihydro-9 (I L-lyxityl) isoalloxazine lyxoflavin, and ethyl-2-keto-3-carboxy-5,6 (4,5-dimethylbenzo) N-ribityl pyrazine. None stimulated vitamin B_{12} production.

As previously noted, the fact that the addition of a compound to inoculated medium results in an increase in B_{12} content over that in the unsupplemented broth does not conclusively prove that this adjuvant is acting as a precursor, even if this increase is a marked one. Indeed this does not even prove that there has been an increase in the amount of the B_{12} produced. *A priori* this apparent stimulation can be attributed to the stimulating compounds acting in one or more of the following ways, namely, a) by increasing the amount of growth, b) by having a sparing action of the B_{12}, thus increasing the response of the assay organism to the level of B_{12} produced, c) by educing a growth response in the assay organism, d) by decreasing the rate of B_{12} destruction, e) by acting as a precursor, and f) by acting as a competitive inhibitor.

It can be proved experimentally that addition of these compounds does not bring about a marked increase in growth. This is particularly pertinent because in these systems growth and B_{12} production are closely correlated. It can also be proved that the compounds do not invoke a growth response with the assay organism. The possibility that these stimulating compounds were able to exert a sparing action on B_{12} was a distinct one in view of the report by Hendlin and Soars (**17**) that o-phenylenediamine, o-xylene, and aniline had a B_{12}-sparing action with *Lactobacillus lactis*. Our results, however, showed no such sparing action with *Escherichia coli*.

The mode of action of these compounds could possibly lie in their inhibition of B_{12} destruction. It must be understood that the assay level of a fermentation broth represents the amount of product in the broth at time of assay, that is the amount of product produced in excess of the amount that is destroyed. Any decrease in the rate of destruction will result in an increase in the broth level. Some light could be thrown on this point if *Streptomyces griseus* could be shown to actively destroy or not to destroy B_{12}. It can be demonstrated experimentally that there is not a marked decrease in the level of B_{12} added to growing cells of *S. griseus*. This, however, is not conclusive proof that the added vitamin is not being destroyed. A non-producing mutant of *S. griseus* does not destroy B_{12}, but here there is the possibility that the capacity

for destruction of B_{12} was lost at the time that the ability to synthesize the compound was lost. This possible mode of action then is difficult to evaluate. Our data would appear to indicate, however, that the rate of B_{12} destruction, if any, is not great enough to account for the marked and rapid increase in B_{12} even if this destruction was completely terminated.

The ultimate proof of precursor activity lies in determining whether labeled compounds are incorporated into the molecule in question. This method was applied by the use of 1,4,5,6-deutero-o-phenylenediamine. Assuming the compound was incorporated into the B_{12} molecule, the deuterium in the 5 and 6 positions would be replaced by methyls. It would also appear to be possible that the o-phenylene diamine could be incorporated into the molecule without being methylated with the resultant formation of a compound different from vitamin B_{12}. The results, however, make it obvious that the o-phenylenediamine, if it is being incorporated into the B_{12} molecule, is being introduced into no more than 20% of the vitamin B_{12} produced (**24**). This certainly does not explain the remarkable enhancing activity of the compound. Hence, a mode of action other than that of a precursor must be sought.

The most probable route of action of the stimulatory compounds lies in their being competitive inhibitors. Competitive inhibition has many permutations, however, and the exact mode of action is obscure. All of the stimulatory compounds are most effective at a level at which they are slightly inhibitory. For example, o-phenylenediamine is effective at a level of 50 γ/ml but is most effective at 100–200 γ/ml or the level at which growth at first is retarded somewhat, then the inhibition is overcome. Under such conditions, use of o-phenylenediamine at a level of 100 γ/ml results in an increase in B_{12} broth potency of 100% (3000 u./ml) and sometimes more. Increases of 200% have been obtained. Additions of o-phenylenediamine to three-day-old fermentations, when growth is near a maximum, has no inhibitory effect and little or no effect on B_{12} production. o-Xylidine and 3,4-diaminotoluene are also most active at a concentration of approximately 100 γ/ml o-Nitroaniline, however, is much more inhibitory and

cannot be used at a level much above 10 γ/ml. These latter three compounds, however, are only approximately 40% as effective as o-phenylenediamine in increasing B_{12} broth potency.

It would be enlightening if the inhibitory and stimulatory actions of the compounds could be separated. The inhibition by o-phenylenediamine may be overcome by the organism in several ways. An enzymatic adaption could be involved. Likewise a compound (B_{12} ?) antagonistic to o-phenylenediamine could be produced by the growing cells. Certainly higher levels of o-phenylenediamine are tolerated if vitamin B_{12} is added at the start of the fermentation.

The o-phenylenediamine may be acting as a selective agent, allowing the resistant nuclei to develop. If this is true, resistant nuclei would have to be very numerous and the mutation to resistance quite common to explain the rapid reversal of inhibition. In addition attempts to select for resistance to o-phenylenediamine met with little or no success though these attempts were not extensive. Such resistant strains, if available, would aid in determining if increased resistance is accompanied by increasing response to increasingly higher levels of o-phenylenediamine.

The response to o-phenylenediamine is not limited to the strains of *S. griseus* with which we have been working nor is it found in all other species of *Streptomyces*. Of some 13 different vitamin B_{12}-producing *Streptomyces* species tested, three gave a marked response to o-phenylenediamine.

Growth and vitamin B_{12} production. It has been noted above that there is an apparent relationship between growth and vitamin B_{12} production. This can be demonstrated by the use of a simple synthetic medium containing a single sugar, such as glucose or maltose, in combination with a single nitrogen source such as $(NH_4)_2HPO_4$, proline, histidine, alanine, or glycine. If one uses an excess of sugar and varies the level of nitrogen from a very low concentration of about 0.2 mg/N ml to an excess of that required for maximum growth, the correlation between growth and B_{12} production becomes apparent.

As is to be expected, the dry-cell weight increases with increasing nitrogen level until a maximum cell weight is obtained. The

vitamin B_{12} level also increases with increasing nitrogen level and with increasing cell weight, the maximum B_{12} level occurring at the time of maximum cell weight. Addition of nitrogen in excess of that required for maximum cell weight does not result in increased production of B_{12} but may bring about lower cell weights with a corresponding decrease in vitamin B_{12} production.

The ratio of B_{12} produced to that of the dry-cell weight does not change appreciably, however, with changing nitrogen level of the medium. Indeed the highest production of B_{12} per mg dry weight of cells usually occurs in the medium with the very low nitrogen levels.

SUMMARY

Streptomyces griseus is capable of producing vitamin B_{12} in a simple synthetic medium containing glucose, $(NH_4)_2HPO_4$ and other inorganic salts. Alanine, glycine, histidine, and proline can be substituted, singly, for $(NH_4)_2HPO_4$. Lactose, maltose, dextrin, starch, and mannitol are suitable as substitutes for glucose.

A large number of complex organic materials, amino acids, carboxylic acids, purines, pyrimidines, and degradation products of vitamin B_{12} were tested for stimulation of vitamin B_{12} production. None induced a stimulation.

Ortho-phenylenediamine, ortho-nitroaniline, ortho-xylidine, and 3,4-diaminotoluene when used as single substituents invoked a significant increase in vitamin B_{12} broth potency.

The most active compound, ortho-phenylenediamine is not a precursor. It possibly acts as a competitive inhibitor.

There is a close correlation between growth and vitamin B_{12} production.

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RESEARCH LABORATORIES,
MERCK & CO., INC.,
RAHWAY, NEW JERSEY

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THE EFFECT OF ENVIRONMENTAL TEMPERATURE ON THE COURSE OF SYSTEMIC MONILIASIS IN MICE[1]

GEORGE H. SCHERR

(WITH 1 FIGURE)

It had become evident to the author that mice infected with moniliasis during the hot summer months displayed a much more severe infection and higher death rate than those infected under more moderate climatic conditions. The following experiments performed under controlled temperature conditions demonstrate the significant role of environmental temperature in the pathogenesis of moniliasis for mice.

MATERIALS AND METHODS

The mice used were a white Swiss strain, departmental stock. All animals received tap water and Purina chow pellets *ad libitum* under both normal and experimental conditions.

Candida albicans strain *171D* has been described in a previous paper (**3**).

The medium used was a broth employed by Reilly, Harris, and Waksman (**2**). Its composition is glucose, 1.0%; peptone, 0.5%; beef extract, 0.5%; NaCl, 0.5%; distilled water. Thirty ml of broth contained in a 125 ml Erlenmeyer was loop-inoculated with *C. albicans 171D* and incubated at 30° C. The centrifuged pellet from such a 48-hour culture was suspended in 16 ml. of sterile saline. This final cell suspension contained 1.1×10^9 cells/ml as determined by plate count.

[1] The work reported here was originally performed by the author at the Department of Bacteriology of the University of Kentucky. The data confirming and extending this temperature effect has since been done as part of a study supported by a contract between the Office of Naval Research, Department of the Navy, and the Creighton University School of Medicine.

Cages containing 10 male mice each were placed in incubators whose temperature ranges were 5°–12° C, 15°–20° C, 28°–32° C, and 35°–37° C. Each mouse received a single infecting dose of 0.2 ml of the *Candida* cell suspension intraperitoneally 24 hours after being placed in the incubator. Uninfected controls were similarly incubated. At the end of 30 days all surviving animals were autopsied.

The degree of dissemination of moniliasis was gauged from the number, size, and distribution of the lesions. As a rule the condition of the kidneys is an excellent indicator of the severity of the infection. The degree of dissemination was assigned a numerical value, as follows:

0.....no visible pathology
1.....few, isolated lesions
2.....frequent lesions
3.....many lesions
4.....marked dissemination of lesions
5.....very marked dissemination

If the animal died, presumably as a result of *Candida* dissemination, prior to the end of the exposure period, a value 1 was added to the base value 5 for every day intervening between death and the termination of the experiment. For example, if an animal died 3 days before the experiment culminated, the dissemination value was designated by the number 8. Thus, the dissemination values recorded here are a measure of both severity of infection and mortality rate. No dissemination values are given for those animals which were found mutilated or were killed accidentally; they are represented by a dash (–) in the various tables.

RESULTS

The data for this experiment are compiled in TABLE I and graphically illustrated in FIG. 1. The temperature ranges of 15°–20° C and 28°–32° C showed the lowest mean dissemination values, 14.2 and 13.8, respectively. A cold temperature (5°–12° C) enhanced the severity of infection manifest at moderate temperatures. The validity of the difference between the mean dissemination values found at cold and moderate temperatures was supported by the statistical analysis of *t*. An environmental temperature of 35°–37°

TABLE I

THE EFFECT OF ENVIRONMENTAL TEMPERATURE ON MICE SYSTEMICALLY INFECTED WITH *Candida albicans 171D*

(Duration of experiment—30 days)

Incubation temp. (°C)	Dissemination values	Infected mice		Dissemination values Controls uninfected
	3	Mean	24.3	—
	16	Av. dev. from mean	7.2	0
	18	Stand. dev. from mean	8.7	0
	27			0
5–12	27			0
	30			0
	30			0
	30			0
	31			0
	31			0
	3	Mean	14.2	
	3	Av. dev. from mean	11.8	0
	4	Stand. dev. from mean	12.3	0
	4			0
15–20	5	*t* for 15°–20° compared to		0
	7	5° C	2.0	0
	26	Degrees of freedom	18.0	0
	27	P	.025–.05	0
	30			0
	33			0
	2	Mean	13.8	0
	3	Av. dev. from mean	11.0	0
	3	Stand. dev. from mean	12.0	0
	3			0
28–32	3			0
	16			0
	19			0
	24			0
	32			0
	33			0
	27	Mean	28.2	—
	27	Av. dev. from mean	1.7	0
	27	Stand. dev. from mean	1.8	0
	27			0
35–37	27	*t* for 15°–20° C compared to		0
	27	35°–37° C	3.38	0
	27	Degrees of freedom	18	0
	31	P	<.005	0
	31			0
	31			0

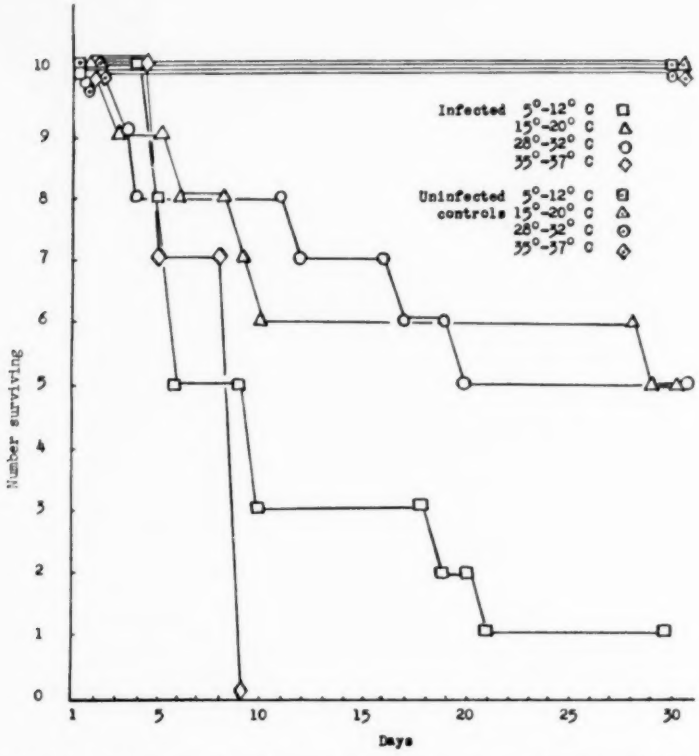

FIG. 1. The effect of environmental temperature on mice systemically infected with *Candida albicans* 1710

C was more detrimental to these infected mice than any of the other temperature ranges studied. In marked contrast to the 50% mortality found at both moderate temperature ranges after 30 days, 100% of the infected animals kept at 35°–37° C were dead after only 9 days.

Uninfected controls kept at the same temperatures as the experimental groups of mice showed no untoward effects.

DISCUSSION

This temperature effect for moniliasis has been confirmed by other experiments. In addition, it has been possible to correlate

sudden peaks of high mortality among *Candida*-infected mice in our colonies with peaks of high temperature occurring during the course of the summer. This enhancement of moniliasis by high environmental temperatures complicates experimental work still further if studies are being conducted with such compounds as cortisone, whose toxicity for mice is also markedly affected by changes in environmental temperature (unpublished data of author).

Kuhn (**1**) found that Swiss mice infected with *Cryptococcus neoformans* that were kept at 35°–36° C showed a higher rate of survival than controls kept at 24°–27° C. Data for other deep mycotic infections with respect to this temperature effect might profitably be collected and assessed as a potential factor affecting the pathogenesis of these diseases. A clinical application of this temperature effect is conceivable but must be preceded by additional laboratory work.

SUMMARY

Swiss mice systemically infected with moniliasis showed a more severe dissemination of the infection and higher death rate when kept at controlled temperatures of 5°–12° C and 35°–37° C than at temperatures of 15°–20° C and 28°–32 C. Uninfected animals kept at the same temperatures as the experimental animals were apparently unaffected. The significance of increases of environmental temperature on the pathogenesis of moniliasis is discussed.

DEPARTMENT OF MICROBIOLOGY,
THE CREIGHTON UNIVERSITY SCHOOL OF MEDICINE,
OMAHA, NEBRASKA

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NOTES ON THE GENUS CAMPTOMERIS, FUNGI IMPERFECTI

ERNST A. BESSEY

(WITH 34 FIGURES)

In 1927, H. Sydow described under the name *Camptomeris calliandrae* Sydow a new genus and species of dematiaceous Fungi Imperfecti, collected by him in Costa Rica on the living leaflets of *Calliandra similis* Sprague and Riley (Family Mimosaceae). Three years later the same author gave the name *Camptomeris leucaenae* (Stevens and Dalbey) Sydow to a fungus collected by him in Venezuela upon the living leaflets of *Leucaena glauca* (L.) Bentham, also one of the Mimosaceae. He compared his fungus with specimens collected by F. L. Stevens in Puerto Rico on the same host and described (by Stevens and Dalbey, **1919**) as *Exosporium leucaenae* Stevens and Dalbey, and recognized that his own collections from Venezuela were identical with those of Stevens from Puerto Rico and belonged to his own previously described genus *Camptomeris*. In 1933 R. Ciferri described another species of this genus collected by him and Ekman in Santo Domingo upon *Desmanthus virgatus* (L.) Willdenow (also one of the Mimosaceae), calling it *Camptomeris desmanthi* Ciferri. Confusion was brought about when, a year later, Petrak (**1934**) redescribed this same fungus, collected in the same locality by Ekman, on the same host, calling it *C. desmanthi* (Ellis and Kellerman) Petrak. He based the change in the authority for the name on his claim that this is the same fungus that was described by Ellis and Kellerman (**1885**) from Kansas on *Desmanthus brachylobus* Bentham (now called *D. illinoensis* (Michx.) MacM.) as *Cercospora condensata* Ell. and Kell. var. *desmanthi* Ell. and Kell. This name was changed by Ellis and Everhart (**1887**) to *Cercospora desmanthi* (Ell. & Kell.) Ell. & Ev. The present author has studied the type of this latter species and has demonstrated that it is generically and specifi-

cally distinct from *Camptomeris desmanthi* Ciferri, of which typical material was also studied. In **1943** C. G. Hanford reported the occurrence of a fungus on *Albizzia coriaria* Welw. from Uganda, East Africa, under the name *Camptomeris albizziae* (Petch) Mason, its identity with *Helminthosporium albizziae* Petch, from Ceylon, having been noted on the herbarium sheet by E. W. Mason. In the herbarium of the Commonwealth Mycological Institute, in Kew, England, are specimens identified on the labels as belonging to *Camptomeris*, but not yet described under this name. Both are on species of *Acacia*, one from Jamaica and one from Natal, South Africa. In southern India, on *Albizzia lebbek* (Willd.) Benth., the same host as that of Petch's species from Ceylon, M. J. Thirumalachar collected a fungus which he published (**1950**) as *Helminthosporium albizzicolum* Thirumalachar and Narasimhan. Examination by the writer of type specimens reveals that this, too, probably should be called a species of *Camptomeris*. At Fort Myers, Florida, in December, 1950, the writer collected another unnamed species of this genus upon *Pithecellobium unguis-cati* (L.) Benth., for which the name *C. floridana* Bessey is to be proposed. Hansford (**1943**) described *C. tephrosiae* Hansf., on *Tephrosia candida* DC., from Uganda, but, as will be indicated further on, this does not seem to belong to *Camptomeris* as the writer delimits it. Except for this species, the hosts of *Camptomeris* all belong to the Mimosaceae and are all tropical or subtropical in their distribution. In 1951 Ciferri proposed the transferral of two other species of fungi to the genus *Camptomeris: Fusicladium robiniae* Shear on *Robinia pseudacacia* L. and *Septoglocum acaciae* Syd. on *Acacia arabica* Willd. In the fuller discussion of the species of *Camptomeris* will be given the writer's reasons for doubting the validity of these transfers.

The genus has been collected in Florida and Texas in the United States; and in Costa Rica in Central America; in Venezuela in South America; and in Jamaica, Puerto Rico and Santo Domingo, in the West Indies. In Asia it has been reported from India and Ceylon. It has been found in Africa at such widely separated localities as Sierra Leone, Gold Coast Colony, Uganda, and Natal. It has been described or named in herbaria under the generic names *Campto-*

meris, Exosporium, Helminthosporium, Cercospora, Cercosporidium, Passalora and *Stigmina*. It would appear, therefore, to be a worth while task to study the leaf-inhabiting species assigned to the foregoing genera insofar as they attack hosts belonging to the Mimosaceae.

All of the accepted species of *Camptomeris* are confined to the living leaves of mostly woody Mimosaceae where they form their conidiophores on the under side of the leaf. They do not usually produce strongly noticeable leaf spots. Mostly the upper surface of the leaf shows a slight yellowing, without a strongly colored margin. On the lower surface of the leaf, at distant intervals or closely placed, the fungus usually forms light brown sub-epidermal hypostromata of more or less isodiametric, rather cubical, fairly thin-walled cells. They may force their way through stomatal openings or, if large, may roll back the epidermis to permit the emergence of small, rather flat-topped sporodochia. In the more typical species of the genus (subgenus *Eucamptomeris*) the only cells emerging from the hypostroma are the enlarged, almost colorless, somewhat balloon-shaped megalophyses. From these exposed cells arise the usually dark-brown conidiophores which bear at or near their tips the single conidia. The conidiophores are more or less concentrically arranged, those toward the center being almost isodiametric and more upright, while those nearer the margin are flattened so as to be somewhat spatulate or even spoon-shaped. These marginal conidiophores are more outspread but then curve upward. The conidia, formed one to a conidiophore, are lighter brown in color and oblong in outline or obclavate or obpyriform or even lanceolate. The apical half may taper markedly or very slightly. The apex is rounded. The base is rounded or truncate, with a nearly central scar of attachment. The conidia have 2 to 3 transverse septa, rarely only 1 or 4. Those septa are in some species strongly thickened, in others remain thin. The conidia are often marked by fine longitudinal and transverse parallel lines of blunt or sharp verrucosities, 0.6 to 2 μ apart and 0.6 to 1.5 μ tall. Sometimes these can be observed only by the use of an immersion lens and may show more clearly in spores that are not quite mature, or only on the basal or apical portion of the spores.

The fungus may appear to the naked eye or hand lens like minute sori of rusts, but under the compound microscope its true nature can be recognized.

After studying type or authentic specimens of all of the eight species recognized as valid by the author, the generic characters may be stated as follows: Parasitic within leaves of Mimosaceae but not producing conspicuously margined leaf spots. Conidiophores hypophyllous, arising from a subepidermal, pale-brown or almost hyaline hypostroma which, without rupturing the epidermis, sends through the stoma balloon-like, almost colorless megalophyses, or which bursts through the stoma and adjacent portions of the epidermis as a small or large, low, more or less flattened sporodochium within which several megalophyses develop. On the separate balloon-shaped megalophyses emerging through individual stomata, or at the tops of the megalophyses making up the upper part of the emerged sporodochium, or from cells of varied size in the absence of distinguishable megalophyses, arise the conidiophores. These are slender or spatulate or spoon-shaped, straight or outwardly and upwardly curved. They are darker in color than the cells of the hypostroma. They may be without transverse septa, but in some species are all transversely septate a short distance from the base. At or near the apex is borne the single conidium. These conidiophores differ from those of *Cercospora* in being unbranched, not branching sympodially and forming a somewhat zig-zag structure as in the latter genus. The conidia differ in their shape from those of *Cercospora* and *Helminthosporium*, rarely averaging more than 2.5 to 5 times as long as broad, and usually being only light brown in color. In shape they may be oblong, nearly equal at each end or more often they are broader near the base and obclavate to obpyriform or lanceolate in outline, sometimes with an obtuse tail. The spores may be straight but often are somewhat curved. They are more often 2–3 times transversely septate, but in one species are regularly 1-septate and in one species 3–4-septate. They are conspicuously roughened or the roughenings may be observed with difficulty if at all.

The genus may be divided into two subgenera distinguished as follows:

Eucamptomeris. Conidiophore-bearing cells (megalophyses) emerging through the stomata singly from the subepidermal hypostroma and bearing the conidiophores apically and laterally. Type species of the genus and subgenus, *Camptomeris calliandrae* Sydow. (See FIGS. 3, 4, 6, 7, 10, 11, 13, 14, 16, 17, 18.)

Exosporioides. Hypostroma bursting through the stoma and sometimes rolling back a considerable amount of the epidermis and cuticle and forming a more or less well marked, mostly flat-topped sporodochium. From enlarged cells (megalophyses) arising from the small basal cells or from branching hyphae or from scattered enlarged cells in the sporodochium arise 2 to 10 conidiophores to each enlarged cell, those nearer the center straighter and more isodiametric, those nearer the margin flattened and bowed outward and upward. Mostly in this subgenus the conidiophores are not so definitely transversely septate near the base as in some species of the subgenus *Eucamptomeris*. Type species of the subgenus, *Camptomeris leucaenae* (Stevens and Dalbey) Sydow. (See FIGS. 22, 23, 26, 29, 30.)

The specific characters in the subgenus *Eucamptomeris* are based chiefly upon the shape and size of the megalophyses, the number and size of the conidiophores and the presence or absence of transverse septa, and the shape and size of the conidia. In the subgenus *Exosporioides* the size and shape of the sporodochium, presence or absence of megalophysis-like cells, shape and size of conidiophores and conidia are important characters.

In the key and in the descriptions of the species the term megalophysis is applied to the large cells arising from the hypostroma or occupying a more or less definite layer in the sporodochium, from whose tops and sides arise the conidiophores. The term sporodochium is used for the cushion-like stroma which is the outer part of the hypostroma which has burst through a considerable area of the epidermis, rolling it and the cuticle back. The term sporodochidium is applied to the cluster of conidiophores and other hyphae growing out of the more-or-less balloon-shaped megalophysis pushed out through a stomatal opening leaving the remainder of the hypostroma beneath the epidermis.

Camptomeris was first named as a distinct genus in 1927. Clements and Shear (**1931**) did not recognize its validity as a distinct genus but included it under the genus described by Earle in 1900 under the name *Cercosporidium*. The non-validity of this rests upon the type of conidiophores, which in the latter genus are sympodially branched and zigzag while in *Camptomeris* they are unbranched and bear but a single conidium.

The eight species recognized by the writer are distinguished in the accompanying key which is followed by the detailed descriptions of the species and comments on excluded species and species that have been mentioned in connection with some valid species of *Camptomeris*.

The proper placement of the genus *Camptomeris* in the artificial system of classification of the Fungi Imperfecti raises some questions. Because of the pigmentation of the conidiophores and spores and sometimes of the hypostroma the genus must be placed among the pigmented groups (*i.e.* the "Phaeo" groups). The subgenus *Exosporioides*, with its rather definite sporodochium, would fall naturally into the family Tuberculariaceae. Only by a straining of the definition can the species of the subgenus *Eucamptomeris* be considered as belonging to this family, for the sporodochidium differs fundamentally from a typical sporodochium. Yet the two subgenera seem to be rather closely related. The type of conidiophore, with its single terminal conidium, distinguishes *Camptomeris* from *Cercospora* and *Cercosporidium*, in both of which the conidiophores are of the zigzag type due to the sympodial mode of branching, with several conidia, one at each joint. *Stigmina* shows many similarities to *Camptomeris* but the limitation of the latter to parasitism in the single family Mimosaceae, the occurrence of special enlarged cells (megalophyses) from which arise the conidiophores, and the flattened nature of the outer conidiophores of a cluster seem to be distinct enough to warrant this separation. *Helminthosporium* is much more deeply colored in most species and usually the spores have more numerous septa. Also in general the conidiophore may bear a number of conidia. Many species of *Helminthosporium* may be saprophytes. If there is any close connection between *Camptomeris* and *Helminthosporium* it is

among the leaf parasites of the latter genus possessing rather short conidiophores bearing each a single conidium. What the perfect stage of *Camptomeris* is, if it still exists, has not been suggested. *C. floridana*, the only species of which a recent collection is available, did not grow when inoculated upon standard laboratory culture media suitable for saprophytic and many parasitic fungi.

CAMPTOMERIS SYDOW

Ann. Mycol. **25**: 142, *f.* 6. 1927.

Eucamptomeris subgen. nov.

Megalophyses plus minusve vesiculiformes; ex hypostromate singulatim per stomata emergentes.

Conidiophore-bearing megalophyses more or less balloon-shaped, emerging through the hypostroma singly through the stomata.

Exosporioides subgen. nov.

Hypostroma per stoma et circumiectam epidermidem et cuticulam emergens et sporodochium formans.

Hypostroma bursting through a stroma and rolling back a considerable amount of epidermis and cuticle and forming a more or less well marked sporodochium.

KEY TO THE RECOGNIZED SPECIES

A. Conidiophore-bearing megalophyses more or less balloon-shaped, emerging from the hypostroma singly through the stomata..... Subgenus *Eucamptomeris*

a. From the megalophysis arise only the conidiophores but not any external hyphae connected with other megalophyses.

b. Conidiophores regularly transversely septate near the base.

c. Conidiophores 6–19 (av. 11) per megalophysis, spreading, 26–35 μ (av. 30 μ) in length; conidia 25–53 × 8–16 μ (av. 41 × 12 μ), 3-septate, oblong to broadly obclavate, rarely curved. On *Calliandra*, Costa Rica.....*C. calliandrae*

cc. Conidiophores 10–46 (av. 26) per megalophysis, semi-erect, 26–51 μ (av. 39 μ) in length; conidia 30–70 × 8–12 μ (av. 47 × 10 μ), 3–4-septate, obclavate, gradually tapering to a narrow, blunt apex, straight or curved. On *Albizzia*, Ceylon and Africa..... *C. albizziae*

bb. Conidiophores not septate near base or only occasionally so.

d. Conidiophores 16–82 (av. 60) per megalophysis, spreading, 17–35 μ (av. 26 μ) in length; conidia 26–47 × 7–11 μ (av. 37 × 9 μ), 3-

septate, oblong, usually slightly curved. On *Acacia*. Natal, S. Africa.....*C. verruculosa*

dd. Conidiophores 16–38 (av. 25) per megalophysis, erect or semi-erect, 25–38 μ (av. 32 μ) in length; conidia 35–62 × 9–13 μ (av. 49 × 11.5 μ), 1- or 3-septate, oblong to narrowly ovate or obpyriform, slightly curved. On *Acacia*. Jamaica..*C. martynii*

aa. From the top and side of the megalophysis arise the conidiophores, but from the lower part there grow out, above the epidermis, short hyphae which merge with those from near-by megalophyses to form a sporodochium-like structure which is connected with the hypostroma only by the emergent megalophyses. Conidiophores 16–105 (av. 41) per megalophysis, upright and spreading, transversely septate near the base, 32–46 μ (av. 39 μ) in length for the outer conidiophores, the inner ones 20–30 μ long; conidia 34–48 × 7–11.5 μ (av. 42 × 10 μ); 1- 2- and 3-septate, oblong to obclavate, usually somewhat curved. On *Pithecellobium*, Florida.....*C. floridana*

AA. Hypostroma bursting through a stoma and rolling back a considerable amount of epidermis and cuticle and forming a more or less well marked sporodochium.....Subgenus *Exosporioides*

c. Conidia 1-septate, not or scarcely narrowed toward the apex, 29–49 × 9–12 μ (av. 41.2 × 10 μ); sporodochia 115–235 μ (av. 188 μ) in length, with a near-basal palisade of megalophyses 24–30 × 7.5–12 μ. On *Desmanthus*. Texas and West Indies.....*C. desmanthi*

cc. Conidia 3-(rarely 2-) septate, distinctly narrowed toward apex, 30–59 × 8.3–10.6 μ (av. 47 × 9.5 μ); sporodochia 80–160 μ (av. 114 μ) broad, with oblong or top-shaped megalophyses, 29–43 × 8.6–14.3 μ, borne on coarse, tubular branching hyphae. On *Leucaena*. West Indies and Venezuela.....*C. leucaenae*

ccc. Conidia 3-(rarely 2-) septate, obclavate to obpyriform, the apical cell narrowed to a straight or curved, blunt beak, 20–34 × 6.2–9.3 μ (av. 27.8 × 7.3 μ); sporodochia 22–54 μ in diameter, consisting of a narrow, compacted mass of upright conidiophores from a base of cells 4–5 μ in diameter. On *Albizzia*, India.....*C. albizziicola*

EUCAMPTOMERIS

CAMPTOMERIS CALLIANDRAE Sydow, Ann. Mycol. **25**: 142. *f.* 6. 1927. FIGS. 1–4.

Megalophyses almost hyaline, 14–23 μ (av. 19 μ) in diameter, measuring the broadest portion of the part that has emerged from the stoma. This emergent portion is 10–26 μ (av. 16 μ) in height while the sub-stomatal portion is 5–13 μ (av. 10 μ) in height. The rather dark conidiophores are 6–19 (av. 11) per megalophysis, in one or two series, spreading outward and then curving upward, with one transverse septum near the base, forming a cell about as long as broad. Rarely more than one septum occurs. The outer,

upwardly curving portion of the conidiophore is flattened somewhat so that the radial diameter is considerably less than the tangential, giving a somewhat spatulate appearance when the cluster of spreading conidiophores (sporodochidium) is viewed from above. Because the conidiophores spread so much the megalophysis looks from above like a central hole in the sporodochidium. Conidiophores 26–35 μ (av. 30 μ) in length by 11–16 μ (av. 13 μ) in tangential diameter. Sporodochidia 48–109 μ (av. 75 μ) across, usually scattered or clustered, but not interconnected by hyphae above the epidermis. Conidia borne singly at the apex of the conidiophores, ovoid to nearly cylindrical, considerably or scarcely narrowed toward the obtuse apex, lighter brown than the conidiophores, mostly not constricted at the septa. Septa 3 in the mature conidia, fewer or lacking in the younger spores. Conidia usually slightly curved at the apical one-fourth or one-third, apparently smooth when fully mature, but when immature somewhat roughened, especially near the base as well as the conidiophore itself. Dimensions of conidia 25–53 × 8–16 μ (av. 41 × 12 μ). Distinct spots are not visible on the upper side of the affected leaflet except slightly yellow-green areas without definite margins.

On leaflets of *Calliandra similis* Sprague and Riley. No. 89 of Sydow, Fungi in itinere costaricensi. Descamparados, Costa Rica. Collected by H. Sydow, December 30, 1924. The specimens studied were from the foregoing exsiccatum, lent by the Mycological Herbarium of the U. S. Department of Agriculture, Beltsville, Maryland, through the courtesy of John A. Stevenson.

CAMPTOMERIS ALBIZZIAE (Petch) Mason, apud C. G. Hansford, Proc. Linn. Soc. London. 155th Session, 1942–1943 (Pt. 1): 61, *f. 1–16*. 1943. FIGS. 8–11.

Helminthosporium albizziae Petch, Ann. Roy. Bot. Gard. Paradeniya **4**: 306. 1909.

Megalophyses 9.6–35.2 × 6.4–28.8 μ (av. 19 × 16 μ) in diameter, measuring the portion above the stoma, and in total height, including the portions within and outside the stoma, about 40 μ (probably somewhat contracted because measured from a stained paraffin section). Conidiophores 10–46 (av. 26) per megalophysis, semierect, 26–51 μ (av. 39 μ) in length and 7–8.5 μ across for the inner, not flattened ones, and 10–17 μ in width, for the outer, flattened conidiophores. Sporodochidia 38–162 × 35–118 μ (av. 83 × 73 μ) across, scattered or crowded, not united. Conidia borne singly at

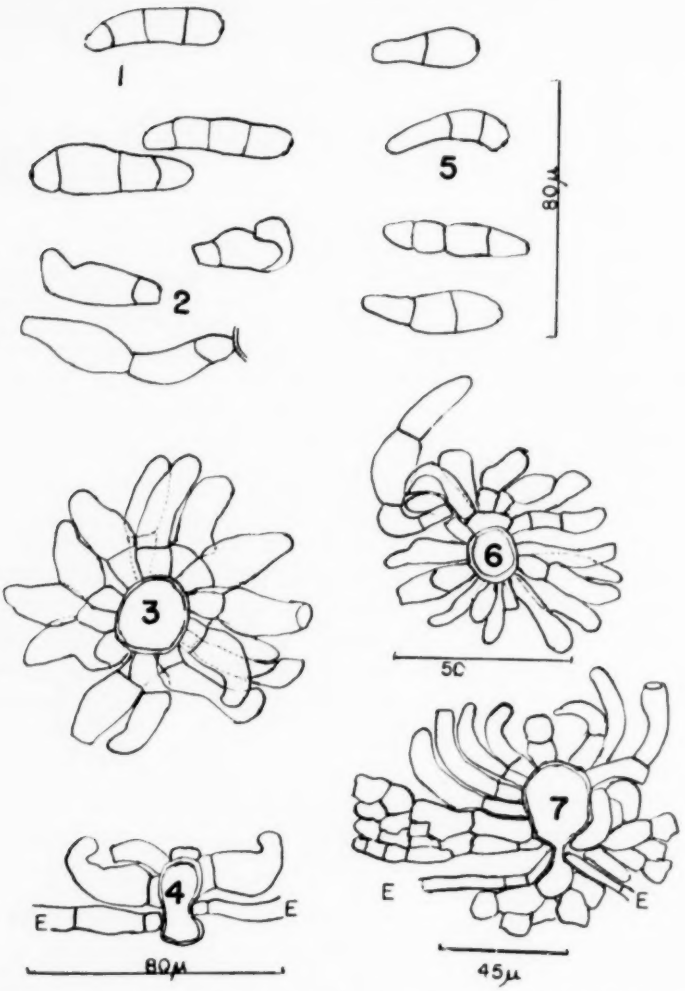
the apex of the conidiophores, obclavate, gradually tapering to a narrow, blunt apex, straight or curved, lighter fuliginous, 30.1–70.4 × 8.3–11.8 μ (av. 47 × 10 μ), 3 to 4 septate (av. 3.5), usually not constricted at the septa, roughened with verrucosities about 1.5 μ apart, less noticeably so on the mature conidia. The dried herbarium specimens (all that were seen) do not show distinctly margined spots on the affected leaves.

Specimens studied: *Helminthosporium albizziae* Petch, type specimen, Petch No. *2589*, on *Albizzia lebbek* (Willd.) Benth., Peradeniya, Ceylon, June 21, 1908 (courtesy Royal Botanical Garden, Kew, England); *Camptomeris albizziae* (Petch) Mason, C. G. Hansford No. *1923*, on *Albizzia grandibracteata* Taubert, Kazi near Kampola, Uganda, Sept. 1936 (courtesy Royal Botanic Garden, Kew, England); C. G. Hansford No. *3458*, on *Albizzia coriaria* Welw., Entebbe Road, Uganda, Sept. 1944 (courtesy of U. S. National Herbarium, through John A. Stevenson); P. Chandler No. *2468*, on *Albizzia* sp., Kampala, Uganda, Sept. 1938 (I.M.I. *7686*, courtesy Commonwealth Mycological Institute); F. C. Deighton No. M. *2591*, on *Albizzia ferruginea* Benth., Njala (Kori), Sierra Leone, Africa, Nov. 28, 1947 (I.M.I. *21151*, courtesy Commonwealth Mycological Institute); S. T. Hughes, on *Albizzia ferruginea* Benth., Gold Coast Colony, Africa (G. C. M. Herb. *1021*), June 13, 1949 (I.M.I. *47542a*, courtesy Commonwealth Mycological Institute).

Camptomeris verruculosa (Sydow) comb. nov. FIGS. 15–18.

Stigmina verruculosa H. & P. Sydow, Ann. Mycol. **10**: 444. 1 *fig.* 1912.

Megalophyses more or less elliptical in cross section, 18–26 × 14–21 μ (av. 22 × 18 μ) in diameter and about 17 μ tall in the portion above the stomatal opening. Conidiophores pale olive-brown, 16–82 (av. about 60) per megalophysis, in about two series, spreading, usually not transversely septate near the base (an occasional conidiophore may be septate), those of the outer series flattened somewhat and slightly curved upward. Conidiophores 17–35 μ (av. 26 μ) in length, in apical view 8.3–11.8 × 6.1–9 μ. Sporodochidia mostly hypophyllous, rarely also epiphyllous, 42–96 μ (av. 71 μ) across, scattered or crowded, but not united. Conidia borne singly at the apex of the conidiophores, oblong, slightly curved,



FIGS. 1–4. *Camptomeris calliandrae* Sydow. 1. Conidia. 2. Conidiophores, one with immature conidium attached. 3. Sporodochidium, from above. 4. Vertical section through megalophysis and attached conidiophores, E. epidermis. FIGS. 5–7. *Camptomeris floridana* Bessey. 5. Conidia. 6. Sporodochidium from below and detached conidiophore with conidium. 7. Vertical section through upper part of hypostroma and megalophysis and attached conidiophores and exostroma, E. epidermis.

26–47 × 7–11 μ (av. 37 × 9 μ), 3-septate (very rarely 2 or 4 septate), not constricted at the septa, pale yellow-brownish. According to Sydow's original description the sporodochidia occupy discolored spots on the leaves.

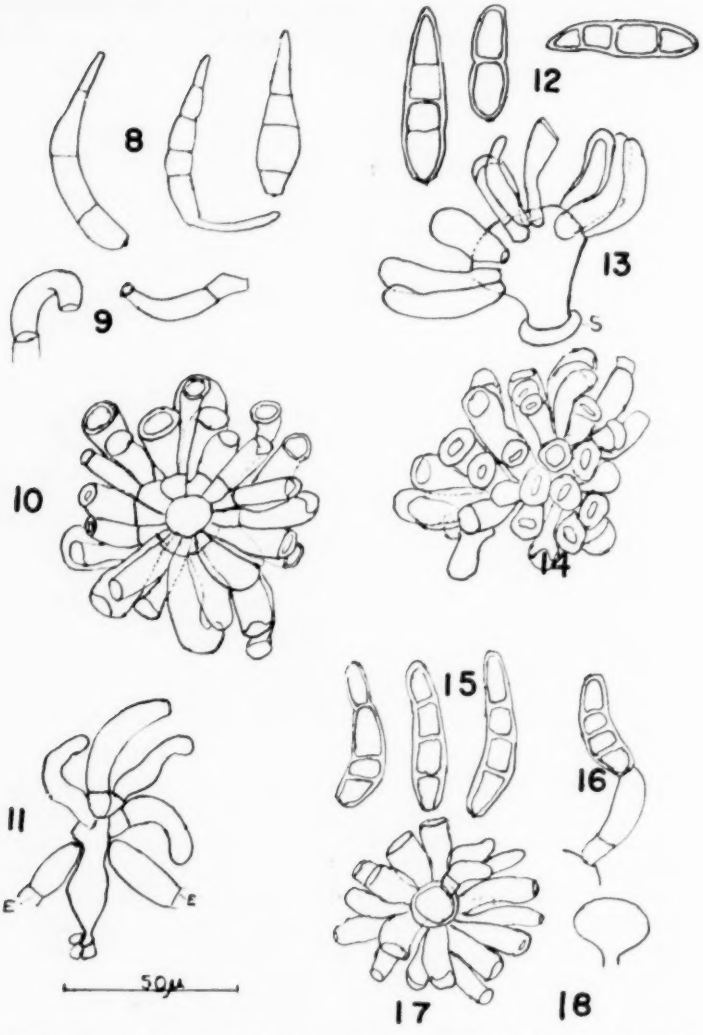
Specimens studied: *Stigmina verruculosa* Sydow (portion of type collection), on *Acacia mollissima* Willd. (= *A. decurrens* Willd.), Harden Heights, Natal, Union of S. Africa, Coll. April 14, 1911 by J. B. Pole Evans, No. *1382* Union of S. Africa Dept. Agr. Myc. Herb. (I.M.I. *38049*, in the *Camptomeris* folder, courtesy Commonwealth Mycological Institute).

Camptomeris martynii sp. nov. FIGS. 12–14.

Megalophyses 12.8–22.4, raro 33 μ (med. 19 μ) diam. in parte suprastomatali et 16–26, raro ad 38 μ (med. 22 μ) alt. supra stoma, fere hyalinae. Conidiophora atro-brunnea, 16–38 (med. 25) in singulis megalophysibus, in pluribus seriebus, erecta seu semi-erecta, exteriora exterius et sursum curvata, et multo applanata, non septata seu raro ad basim septata; 25–38 μ (med. 32 μ) long., exteriora 8–12.8 μ (med. 10 μ) lat., interiora 4.8–9 μ (med. 7 μ) lat. Sporodochidia hypophylla, 55–104 μ (med. 74 μ) lat., sparsa vel aggregata. Conidia singulatim orta ad apices conidiophororum, oblonga seu anguste obpyriformia, e medio ad obtusum apicem angustata, plerumque 3-septata vel frequenter 1-septata, medio septo crassiore quam dua alia, interdum ad septum medium constricta, praecipue conidia immatura exigue verruculosa, pallide brunnea, interdum paululo curvata in dimidio apicali, 35–62 × 9.2–12.8 μ (med. 49 × 11.5 μ).

In foliis vivis *Acaciae villosae* (Sw.) Willd. Long Mountain, Jamaica. Coll. E. B. Martyn, 1949.

Megalophyses 12.8–22.4 μ, rarely 33 μ (av. 19 μ) in greatest diameter of the extrastomatal portion and 16–26, rarely up to 38 μ (av. 22 μ) in height above the stoma, almost hyaline. Conidiophores dark brown, 16–38 (av. 25) per megalophysis, in several series, erect or semi-erect, the outer ones curved outward and upward and flattened considerably, usually not septate but occasionally one transverse septum near the base. Conidiophores 25–38 μ (av. 32 μ) in length, those nearer the margin 8–12.8 μ (av. 10 μ) in breadth, the more centrally placed ones 4.8–9 μ (av. 7 μ) across. Sporodochidia hypophyllous, 55–104 μ (av. 74 μ) across, usually scattered, not united even when crowded. Conidia borne singly at the apex of the conidiophore, oblong to narrowly obpyriform, usually narowed from the middle to the blunt apex. Mostly 3-, or often 1-, septate, the middle septum much thicker than the other two, sometimes constricted at the middle septum; very faintly roughened, especially when immature, pale brown, occasionally



FIGS. 8–11. *Camptomeris albizziae* (Petch) Mason. 8. Conidia, one with basal germ tube. 9. Conidiophores. 10. Sporodochidium from above. 11. Megalophysis and attached conidiophores, E. epidermis. FIGS. 12–14. *Camptomeris martynii* Bessey. 12. Conidia. 13. Megalophysis with attached conidiophores, S. stoma. 14. Sporodochidium from above. FIGS. 15–18. *Camptomeris verruculosa* (Syd.) Bessey. 15. Conidia. 16. Conidiophore attached to megalophysis and bearing a conidium. 17. Sporodochidium, from below. 18. Side view of the portion of a megalophysis extending above the stomatal opening.

slightly curved, especially in the apical half; 35–62 × 9.6–12.8 μ (av. 49 × 1.5 μ). No conspicuous spot produced on the affected leaflets so far as visible on the dry herbarium specimen. Named in honor of E. B. Martyn, collector of the type material.

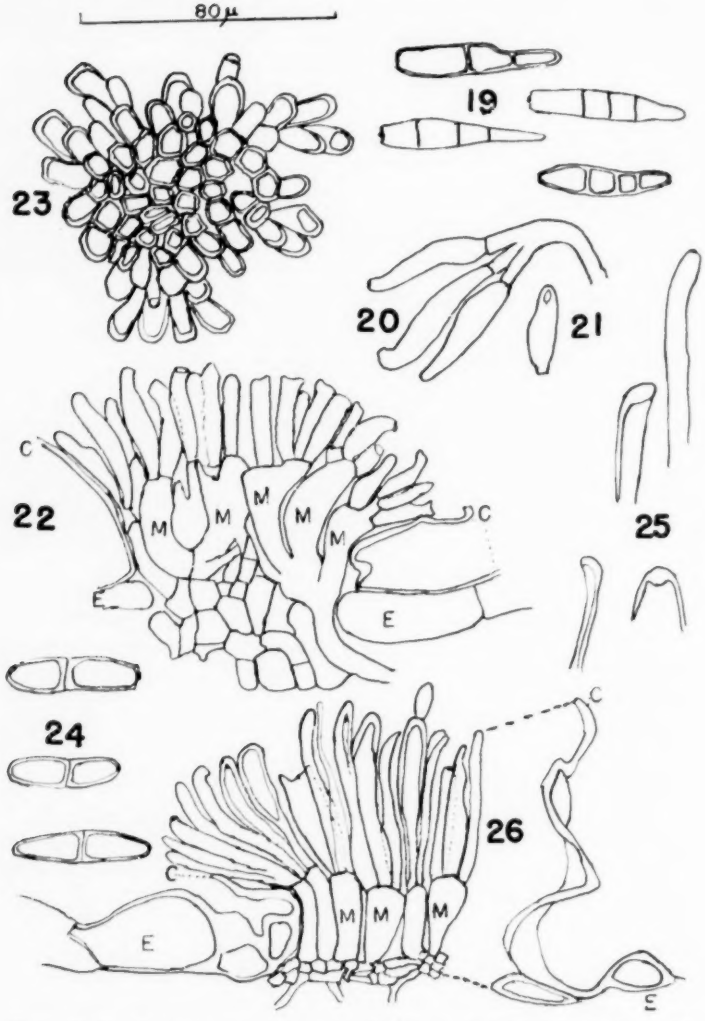
On leaflets of *Acacia villosa* (Sw.) Willd., Long Mountain, Jamaica. Collected by E. B. Martyn, February, 1949. Department of Agriculture, Jamaica, No. P. *18426* (I.M.I. *34795*, courtesy Commonwealth Mycological Institute).

Specimen studied, the foregoing type collection (which is placed in the *Camptomeris* folder, without trivial name).

Camptomeris floridana sp. nov. FIGS. 5–7.

Maculae in foliis hospitis inconspicuae. Sporodochidia hypophylla, raro epiphylla, singulatim vel densiuscule dispersa, subatra, minuta, 86–109 μ diam. Hypostroma pallide ferrugineum, circa 100 μ diam., 25–50 μ crassum. Cellulae hypostromatis pseudoparenchymaticae, 6–14 μ diam., quarum media cellula (megalophysis) sub ostiolo situata, paululo maior. Haec cellula crescit ex ostiolo stomatis et fit vesica 22–42 μ alt. et 18–26 μ lat. Ex huius cellulae superficie oriuntur radiatim multae breves cellulae quarum superiores ferunt conidiophora et ex inferioribus oritur ectostroma. Conidiophora recta seu plus minusve sursum arcuata, cylindracea, 20–46 μ long., 3.5–7.5 μ lat., apicem versus plus minusve attenuata. Conidia singula, acrogena, obclavata vel cylindracea, ad basim rotundata, sursum paululo attenuata, plerumque varie curvata, dilute olivaceo-brunnea, 1–3 septata, ad septa vix constricta, 34–48 × 8–11.5 μ, minute asperulata. Hab. in foliis *Pithecellobii unguis-cati* (L.) Benth. Ft. Myers, Florida, U. S. A. 30. XII. 1950. Leg. Ernst A. Bessey.

Megalophyses 18–26 μ (av. 23 μ) in greatest diameter above the stoma; 22–42 μ (av. 32 μ) in height in the supraepidermal portion, and 16–26 μ in the subepidermal part, the almost hyaline wall of the external portion 1.6–2.5 μ thick. The light brown hypostroma of pseudoparenchymatous tissue is made up of thin walled cells 6–14 μ in diameter and is up to 100 μ or more in width and projects 25–50 μ into the mesophyll, with a few very fine, hyaline hyphae extending to the upper epidermis. The host cells, except where crushed by the stromatic tissue, appear to remain alive although their walls are browned, often clear to the upper epidermis. From the external portion of the megalophysis arise numerous, dark, closely packed cells. Those arising nearer the epidermis multiply and form a mass of stromatic tissue or sporodochium, which remains free from the epidermis. Spreading laterally it joins with similar masses of cells from other megalophyses near by. The cells growing from the upper half of the megalophysis give



FIGS. 19–23. *Camptomeris leucaenae* (Stevens & Dalbey) Sydow. 19. Conidia. 20. Branch of sporodochium bearing three conidiophores. 21. Conidiophore. 22. Vertical section through sporodochium, C. cuticle, E. epidermis, M. Megalophyses bearing conidiophores. 23. Sporodochium from above. FIGS. 24–26. *Camptomeris desmanthi* Ciferri. 24. Conidia. 25. Upper portion of conidiophores. 26. Vertical section through sporodochium, C. cuticle, E. epidermis, M. Megalophyses bearing conidiophores.

rise to the conidiophores, one or rarely two or even three arising from each of these cells. In the cases where only one conidiophore develops the basal cell probably is homologous to the basal cell of the conidiophore as found in other species of *Camptomeris*. The conidiophores from nearer the top of the megalophysis are shorter than those arising from the sides. Inner conidiophores 20–30 μ in length and 3.5–7.5 μ in thickness, sometimes slightly more slender toward the apex, the outer ones 32–46 μ (av. 39 μ) long. They are curved upward and inward. Each bears a single apical conidium. The sporodochidia all arise independently and may be scattered but the ectostromata of those arising closer together join to form a united group of 2 to 3 or even 8 to 10. Rarely a few scattered sporodochidia are produced on the upper side of the leaflet. The width of the individual sporodochidia varies from 86–109 μ (av. 99 μ). From above, the megalophysis is sometimes visible but when the sporodochidia are scraped off by a scalpel and viewed from the under side it is very noticeable as an almost round hole. The conidiophores are lighter in color than their supporting cells but are darker than the conidia. Conidia oblong to obclavate, usually somewhat curved, transversely 1- 2- and 3-septate, the septa thin; minutely verruculose, the verrucosities about 1 μ apart in transverse and longitudinal parallel rows about 1 μ apart. 1-septate conidia 34–42 × 9.6–11.5 μ (av. 37 × 10 μ); 2-septate 42–45 × 7–11.2 μ (av. 43 × 10 μ); 3-septate 42–48 × 8–11.5 μ (av. 46 × 10 μ). These form respectively 40, 25, and 35 percent of the total number of the conidia measured. The living, affected host leaflets show from above no definite spots or sometimes a slight yellowish-green discoloration. From below, the numerous sporodochidia appear black to the naked eye and even when viewed through a hand lens resemble very small rust sori.

On leaflets of *Pithecellobium unguis-cati* (L.) Benth. Coll. Ernst A. Bessey, Ft. Myers, Florida. Dec. 30, 1950. Only specimens studied were from the foregoing type collection.

The type collection has been divided and deposited in the following herbaria: Michigan State College, University of Florida, Mycological Herbarium of the U. S. Department of Agriculture, Commonwealth Mycological Institute, New York Botanical Garden.

EXOSPORIOIDES

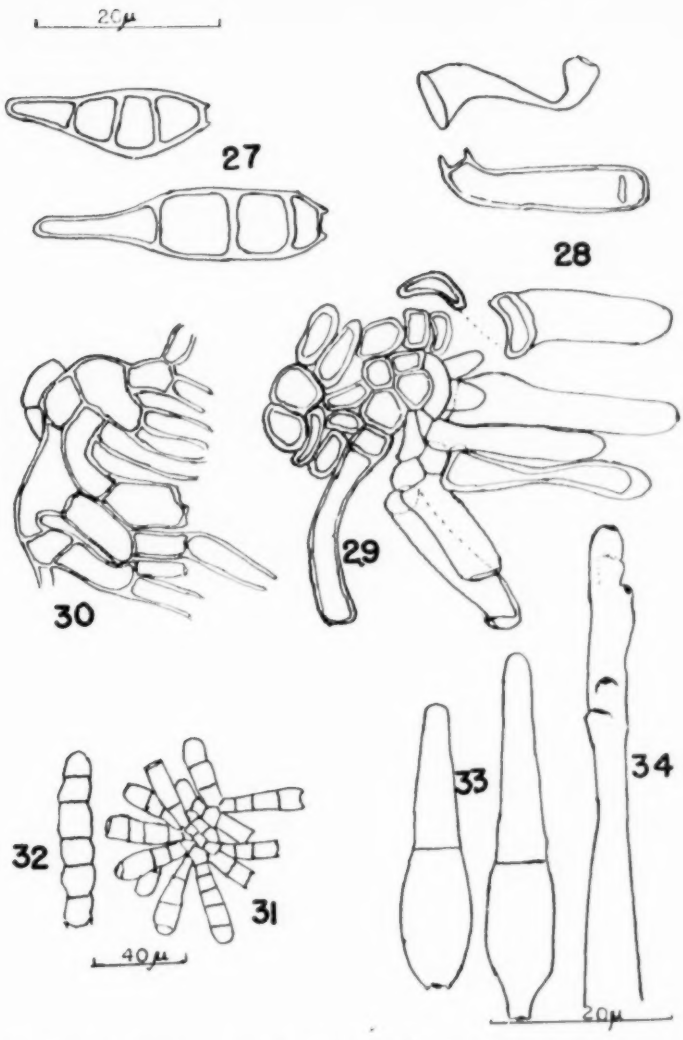
CAMPTOMERIS DESMANTHI Ciferri, Ann. Mycol. **31**: 165. 1933. FIGS. 24–26.

Numerous dark brown sporodochia in hypophyllous patches 2–5 mm. across, on the not conspicuously discolored leaflets. The

sporodochia are elongated in the direction of the main axis of the stomata through which they first burst and measure 115–235 × 100–166 μ (av. 188 × 152 μ). They form rather flat cushions, the upper layer made up almost entirely of the conidiophores. Sections made from paraffin-embedded leaflets show that subepidermally there is a low hypostroma from the upper surface of which arises a layer, more or less palisade-like, of a few up to many enlarged cells (megalophyses) narrowly balloon shaped, measuring 24–30 × 7.5–12 μ. From the upper rounded or flat surface of each megalophysis arise 5 to 6, even up to 10 conidiophores, so that the sporodochium that bursts through the epidermis may be compared to a compound structure of several to many sporodochidia, such as are formed and emerge singly in *Camptomeris calliandrae* and other species in the subgenus *Eucamptomeris*. The conidiophores at the outer edge of each of these small megalophyses curve outward and upward, those nearer the center being less curved and more upright. The conidiophores are dark brown, 45–61 μ in length, those not at the margin being usually 9.6–10.6 × 4.5–7.4 μ (av. 10 × 6 μ) in end view while the marginal ones may be spoon-shaped and in end view up to 13 × 3 μ. They may be somewhat hooked. They bear but one conidium each. They may have a transverse septum near the base forming a cell about 6 μ long and 5 μ broad, but this septum seems to be lacking in many conidiophores (and is not shown in the vertical section of the sporodochium in Fig. 26). The conidia are lighter brown, oblong, usually 1-septate (only one was found with 3 septa). They are usually, but not always, constricted a little at the septum. The septum is 2–4 μ thick, the outer wall of the spore being not so thick. The apical and basal halves of the conidia are quite similar and rounded at the ends. Rarely the distal half is somewhat tapering. They measure 29.4–48.6 × 9–11.8 μ (av. 41 × 10 μ). They are very minutely striate, transversely and longitudinally, with rows of very small verrucosities, visible mostly only under the oil-immersion. The foregoing measurements and the illustrations were all made from the type collection. From W. H. Long No. *6508*, collected at Brownsville, Texas, the conidia measured 34.6–45.8 × 8.6–12 μ (av. 40 × 10 μ).

On *Desmanthus virgatus* (L.) Willd., Valle de Cibao, prov. Santiago, Santiago, Hato del Yaque, Dec. 31, 1930, coll. E. L. Ekman and R. Ciferri.

Camptomeris desmanthi (Ell. & Kell.) Petrak. Petrak, F. Mykologische Notizien XII. No. *762*. Über *Cercospora desmanthi* Ell. & Kell. Ann. Mycol. **32**: 333. 1934, was described from specimens collected by E. L. Ekman in Santo Domingo on



FIGS. 27–30. *Camptomeris albizziicola* (Thirumalachar & Narasimhan) Bessey. *27*. Conidia. *28*. Conidiophores. *29*. Portion of sporodochium from above, with a few attached and detached marginal conidiophores. *30*. Vertical section through a portion of a sporodochium. FIGS. 31–32. *Camptomeris tephrosiae* Hansford (after Hansford, 1943). *31*. Cluster of conidiophores from above. *32*. Conidium. FIGS. 33–34. *Cercospora condensata* var. *desmanthi* Ell. & Kell. *33*. Conidia. *34*. Conidiophore. From type specimen.

the same host and in the same locality as Ciferri's type collection. The description matches in the main that given a year earlier by Ciferri, but Petrak erroneously identified his fungus with *Cercospora condensata* Ell. & Kell. var. *desmanthi* Ell. & Kell., described in 1885, but later made a distinct species, *Cercospora desmanthi* (Ell. & Kell.) Ell. & Ev. 1887.

Specimens of *Camptomeris desmanthi* Ciferri studied: Ciferri Mycoflora Domingensis *3899*. Hato del Yaque, Santo Domingo. Coll. Ciferri and Ekman, Dec. 13, 1930. This is the same locality as the type collection. (Courtesy of U. S. Department of Agriculture, Mycological Collection.) Other specimens of this same species studied by the writer were the following, labeled *Cercospora desmanthi* Ell. & Kell. W. H. Long Herb. *6163*, on *Desmanthus velutinus* Scheele, San Antonio, Texas, Nov. 8, 1916, and *6508* on *Desmanthus* sp. from Brownsville, Texas, May *27*, 1918. On the leaflets of a specimen of *Desmanthus leptolobus* T. & G., Lindheimer, Flora Texas *760* collected at Comanche Springs or New Braunfels, Texas, June 1851, recognizable clusters of this fungus were found. (Specimens made available by courtesy of Dr. G. J. Goodman, of the Herbarium of the University of Oklahoma.) Specimens labeled *Cercospora condensata* Ell. & Kell. var. *desmanthi* Ell. & Kell. and *Cer. desmanthi* (Ell. & Kell.) Ell. & Ev. studied by the writer are discussed in greater detail later in this paper.

CAMPTOMERIS LEUCAENAE (Stevens & Dalbey) Sydow, Ann. Mycol. **28**: 222. 1930. FIGS. 19–23.

Exosporium leucaenae Stevens & Dalbey, Mycologia **11**: 5. Pl. 2, figs. 5–7. 1919.

Sporodochia dark brown, scattered, hypophyllous. Leaflets not conspicuously spotted. Sporodochia not noticeably elongated, 80–160 μ (av. 114 μ), forming rather flat cushions, the upper layer made up almost entirely of the conidiophores. From the basal layer of small cells there arise coarse, branching tubular hyphae with swollen oblong or top-shaped ends (megalophyses) 29–43 $\times$ 8.6–14.3 μ. These bear at the flattened top mostly about 3 (sometimes 2 or 4) conidiophores. From the megalophyses nearer the center of the sporodochium the conidiophores are more nearly isodiametric in cross section while from those nearer the margin the conidiophores are more flattened and spatulate or spoon-shaped. In length

they are 32–46μ. Those nearer the margin measure in end view 9.6–10.2 × 6.4–9 μ. The conidia are 2 or 3 septate, more often the latter, the septa and outer walls of the more mature spores somewhat thickened, 30–59 × 8.3–10.6 μ (av. 47 × 9.5 μ). They are obclavate or narrowly lanceolate in outline, distinctly narrowed in the distal portion, very faintly roughened. The above measurements were made on a portion of the type collection.

Specimens studied: *Exosporium leucaenae* Stevens and Dalbey. Portion of the type collection on *Leucaena glauca* (L.) Benth. Porto Rican Fungi *6792*. Coll. F. L. Stevens. Arecibo, P. R., Jan. 17, 1914 (by courtesy of the Herbarium, University of Illinois); *Comptomeris leucaenae* (Stev. & Dalb.) Syd. on *Leucaena glauca* (L.) Benth., collected by H. Sydow, near Caracas, Venezuela, June 2, 1898 (courtesy Mycological Collection of U. S. Department of Agriculture); *3133* of P. Sintinis, Plantae Portoricenses, Coano, in convalli rivuli Moreno, Puerto Rico, Dec. 18, 1885; coll. R. Ciferri, Santiago, Hato del Yaque, August 1931, I.M.I. Herbarium *3026* ex Petrak, Mycotheca generalis *726* (by courtesy of Commonwealth Mycological Institute).

CAMPTOMERIS ALBIZZIICOLA (Thirumalachar & Narasimhan) comb. nov. FIGS. 27–30.

Helminthosporium albizzicolum Thirumalachar & Narasimhan, Sydowia **4**: 67. 1950.

Sporodochia hypophyllous, numerous, scattered or concentrically arranged, in red-brown spots 2 to 4 or 5 mm. × 1 to 2 mm., 22–54 μ (av. about 36 μ) in diameter, about as long as wide. They apparently start to push through a single stoma but burst this and turn back the surrounding epidermis and cuticle. The top is rather flat and from above only the upper portions of the conidiophores and the freed conidia are visible. At the base is a mass of more or less isodiametric or somewhat elongated dark-walled cells, 4 to 5 μ in diameter, from which arise the conidiophores, packed closely together, those in the center rather straight but towards the margin bowed outward and upward. Conidiophores pale brown, 12–25 μ long, the central ones nearly isodiametric, 3–5 μ across, those near the margin flattened and 6–8 × 2–3 μ in cross section. Some of the marginal ones are hooked at the apex where the scar of the single conidium is sometimes very distinct, often subapical. Some conidiophores appear to have a transverse septum near the base

but possibly the cell cut off by this should be regarded as a cell of the basal sporodochium. Conidia more dilutely brown, obpyriform to broadly obclavate, rounded at the base with a distinct scar of attachment, the distal portion narrowed to a straight or curved obtuse beak; 3- (rarely 2-) septate, the septa and wall of the spore rather thin. Under the oil immersion the whole spore or only its basal and apical portion show a very fine aculeation, the aculeae being 0.6–0.7 μ apart and 0.6–1.5 μ high. Conidia 20–33.6 × 6.2–9.3 μ (av. 28 × 7.3 μ).

Specimens studied: Portion of the type collection of *Helminthosporium albizzicolum* on *Albizzia lebbek*, Tirupati, South India. Coll. M. J. Thirumalachar, Jan. 10, 1946 (courtesy of M. J. Thirumalachar).

SPECIES EXCLUDED

CAMPTOMERIS TEPHROSIAE Hansford, Proc. Linn. Soc. London 155th Session, 1942–1943 (part 1): 61. FIG. 12. 1943. FIGS. 31, 32.

Authentic specimens of this fungus could not be located in the Herbarium of the Commonwealth Mycological Institute nor in the Herbarium of Kew Botanic Garden. On information from E. W. Mason of the former, the Uganda Herbarium is at present not accessible. The following descriptions are taken from the original publication by Hansford:

22. Camptomeris Tephrosiae Hansford sp. n. Maculae epiphyllae, flavidae, elongatae, in centro brunneae, hypophyllae, indistinctae, dilute flavo-brunneae. *Sporodochia* plerumque hypophylla, minuta, atra, pulvinata, circa 100 μ diam. *Conidiophora* radianta, plus minusve erecta, olivacco-brunnea, 2–3 septata, haud constricta, simplicia, 30–10 μ, inferne attenuata. *Conidia* clavato-cylindracea, atro-brunnea, levia, 3–5 septata, leniter constricta, basi truncata, apici rotundata, 50–95 × 16–22 μ. Hab. in foliis Tephrosiae candidae, Kampa'a, Hansford 1862.

Causes defoliation of the host; the leaf-spots are lenticular, elongated along the veins of the leaflets. There is little differentiation of the conidiophores and conidia; together each consists of a single row of cells which break off at a septum, the outer wall remaining as a broken ring around the apex of the "conidiophore" and around the base of the "conidium."

By the description and illustration this is certainly not congeneric with *Camptomeris*, but the writer, without opportunity to study au-

thentic specimens, dares not venture to indicate where it belongs. The 2–3-septate conidiophores, the 3–5-septate, clavate-cylindric conidium, which on separation from the conidiophore leaves a broken ring, are characters lacking in typical *Camptomeris*.

CAMPTOMERIS ROBINIAE (Shear) Ciferri, Mycopath. **6**: 25. 1951.
Fusicladium robiniae Shear (1902).

The writer has examined specimens of this fungus collected by J. A. Stevenson and Miss Cash in 1936 at Mountain Lake Biological Station, Virginia, and checked with Shear's type specimen of this fungus, as well as specimens collected by Dr. and Mrs. Shear and distributed in Fungi Columbiani, Century XIII. These showed all the characteristics of *Passalora* and in measurements and other characters of the conidiophores and conidia cannot be distinguished from *P. bacilligera* (Mont. & Fr.) Fr. apud Fres., the type species of the genus, except possibly by the host and the more marked spots on the leaves. This species must, therefore, be excluded from the genus *Camptomeris*.

CAMPTOMERIS ACACIAE (Sydow) Ciferri, Mycopath. **6**: 25. 1951.
Septoglocum acaciae H. Sydow, Ann. Mycol. **12**: 489. 1914.

On *Acacia arabica* Willd. Coimbatore, Madras Pres. India. Coll. W. McRae. Specimens could not be obtained for study. The fact that Sydow states that the conidiophores are lacking ("sporophoris nullis") and that the "acervuli" are subcutaneous and that the spores emerge in light yellow drops would seem to bar this from inclusion in *Camptomeris*.

CERCOSPORA CONDENSATA var. DESMANTHI Ell. & Kell. Journ. Mycol. **1**: 2. 1885.
Cercospora desmanthi (Ell. & Kell.) Ell. & Kell. Jour. Mycol. **3**: 14. 1887.
Cercosporidium desmanthi (Ell. & Kell.) Earle ex Dearness. Mycologia **33**: 365. 1941.

This fungus was described from specimens of *Desmanthus brachylobus* Benth. (more properly *D. illinoensis* (Michx.) MacM.)

collected by W. A. Kellerman at Great Bend, Kansas, August, 1884.

Specimens studied: Kellerman *632*, type specimen, from Great Bend, Kansas, Aug. 1884. Others from Kansas were Kellerman *941*, Manhattan; Kellerman & Swingle, Kansas Fungi *5*; Ellis & Everhart, North American Fungi *1992*; Bartholemew, Fungi of Kansas *1892*; from Missouri, Rabenhorst-Pazschke, Fungi Europaei et extraeuropaei *4192*; from South Dakota, Brenckle Fungi Dakotenses *627*; from Louisiana, Langlois *548*, July 27, 1886. This is the specimen on which the var. *desmanthi* was raised to specific rank; from New Mexico, Earle and Earle, Plants of New Mexico *267*; from Texas, on *Desmanthus acuminatus* Benth., W. H. Long Herbarium *5399* and *6147*; on *D. velutinus* Scheele, W. H. Long Herbarium *6183*. This fungus occurred on a specimen of *Desmanthus illinoensis* in Plants of Oklahoma *5442*, collected in the Arbuckle Mts., Murray Co., Okla.

The compact sporodochia, emerging through the stomatal openings which they soon rupture and greatly enlarge, bear on their flattened surface numerous conidiophores which are compacted together below their free apical portions with measure 35–50 × 3.5–6.2 μ (av. of 5, 43 × 5 μ). These apical portions are not septate, but toward the outer end are somewhat zig-zag, bearing the scars of 4 to 8 spores which have been formed sympodially. In cross section the conidiophores are almost isodiametric. They are not flattened or spoon-shaped, even at the margin of the sporodochium. The conidia are obclavate or narrowly lanceolate in outline, but with a rounded tip. About 67% are 1-septate, the remainder being 3- or 2-septate. They measure 23.1–41.3 × 5.9–8.8 μ (av. 31 × 7.3 μ). The septa and outer wall are not pronouncedly thickened.

Although this fungus possesses the *Cercospora* type of conidiophores, by which it is entirely distinct from *Camptomeris*, the conidia are broadly blunt and only 2.8 to 6.3 times as long as broad at the point of greatest diameter (average ratio of length to breadth 4.3 to 1), whereas in typical *Cercospora* the ratio is 10 to 1 or more. For example, in *Cercospora olivacea* (Berk. & Rav.) Ell., on *Gleditsia triacanthos* L., this ratio as measured by the writer is

8.0–32.5 to 1, averaging 17.1 to 1. The genus *Cercosporidium*, with *C. euphorbiae* (Tracy & Earle) Earle as the type species, was established by Earle (**1900**) to accommodate a fungus with elongated, more or less spreading conidiophores of the sympodial type arising in a sort of sheaf or sporodochium-like structure, but with relatively short conidia. Later a few other species were added and in 1941 Dearness transferred *Cercospora desmanthi* (Ell. & Kell.) Ell. & Ev. to it, crediting the change to Earle. Later, Earle (**1902**) questioned the validity of *Cercosporidium* and transferred its species to the older genus *Passalora*. That this is wrong is made evident by a study of the type species, *P. bacilligera* (Mont. & Fries) Fries ex Fres. The writer has not been able to obtain the type specimen but has studied various exsiccata of this species prepared by such European mycologists as Krieger, Petrak, Tranzschel & Serebrianikow, Kabát & Bubák, Fuckel, Kirulis, Keissler, Sydow, Rabenhorst, Roumeguère, Saccardo, deThümen, Briosi & Cavara, and others, loaned by the generosity of the Mycological Herbarium of the U. S. Department of Agriculture and of the New York Botanical Garden. After a study of these the writer agrees with von Höhnel (**1923**) that *Passalora* is distinguished from *Fusicladium* by the conidiophores of the former breaking out in a bundle through a stomatal opening and after a short distance separating somewhat to form free, mostly unbranched, sparsely, if at all, septate portions 50 to 150 μ long. At the apex of each conidiophore arises a single conidium which is by-passed by the sympodial branching of the conidiophore to bear another conidium, and so on until several, up to 5 or 6 more conidia have been produced, each falling when mature. As a result the apical portion is a little thicker than the main portion of the conidiophore and slightly zigzag and bears the scars of the fallen conidia. The conidia are always uniseptate, rather slender, blunt at the apex, the terminal cell narrower than the somewhat swollen basal cell. The walls and the septum are not much thickened. In *Fusicladium* the conidiophores are formed subcuticularly and are short, unbranched and mostly non-septate, each bearing only a singly apical conidium. Closely related to *Passalora* and distinguished mainly by the number of septa in the conidium is *Cercosporidium*. The type species, *C. euphorbiae* (T.

& E.) Earle (**1900**), was based on *Scolecotrichum euphorbiae* Tracy & Earle (**1896**). This was shown by Shear (**1902**) to be the same as *Fusicladium fasciculatum* Cke. & Ell. (**1878**) and was transferred to *Scolecotrichum* under the name *S. fasciculatum* (C. & E.) Shear. In 1902 Earle concluded that this was congeneric with *Passalora* and gave it the name of *P. fasciculata* (C. & E.) Earle, transferring *C. euphorbiae* (T. & E.) Earle, and *C. helleri* Earle, to *Passalora*. The writer's study of the type collection of *Scolecotrichum euphorbiae* T. & E. revealed that the conidia are 68% 3-septate, 8% 2-septate and 24% 1-septate. The last were in many cases apparently immature. For this reason he agrees with von Höhnel's conclusion that the genus *Cercosporidium* is valid and that the correct name of the type species is *C. fasciculatum* (C. & E.) Höhn. Because one-third of the conidia are more than 1-septate the writer agrees with Dearness (**1941**) in placing *Cercospora desmanthi* (E. & K.) Ell. & Ev. in the genus *Cercosporidium* under the name *Cercosporidium desmanthi* (E. & K.) Earle. It has nothing to do with *Camptomeris desmanthi* Cif. and the name *Camptomeris desmanthi* (E. & K.) Petrak is invalid as it is a later name and is based on two distinct fungi. *Cercospora condensata* E. & K. on *Gleditsia triacanthos* L. and *C. olivacea* (B. & Rav.) Ell. on the same host are typical species of *Cercospora* and have nothing to do with *Cercosporidium*.

The writer would urge all mycologists interested in *Camptomeris* and related fungi to make a careful study of all available specimens that have been described as leaf parasites, especially upon Mimosaceae, and perhaps also upon the related Caesalpiniaceae and Fabaceae and ascribed to the genera *Cercospora, Cercosporidium, Passalora, Helminthosporium, Stigmina,* and *Exosporium*. It is to be expected that other species of *Camptomeris* may be turned up by such a search.

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P. Rogers, who made loans of specimens from the N. Y. Botanical Garden Herbarium, especially type specimens from the J. B. Ellis herbarium; E. W. Mason, for numerous specimens from the herbarium of the Commonwealth Mycological Institute; the Royal Botanic Garden at Kew, for specimens from the Herbarium; Professor Marie B. Morrow, for specimens from the Herbarium of the University of Texas; Dr. Leland Shanor, for type material of *Exosporium leucaenae* from the Herbarium of the University of Illinois; Dr. M. J. Thirumalachar, for a portion of the type collection of *Helminthosporium albizzicolum;* Dr. C. J. Alexopoulos and Charles L. Gilly, my colleagues in Michigan State College, for advice in matters nomenclatorial, and to others who have assisted me by making slides, etc. Thanks are also due to Dr. G. J. Goodman of the Department of Botany of the University of Oklahoma, who permitted the writer to study the Mimosaceae in the herbarium in the search for species of *Camptomeris* and *Cercosporidium*.

Postscript. While awaiting the printing of the manuscript of this paper there appeared the following publication in which the genus *Camptomeris* was treated: Hughes, S. J. Studies on micro-fungi. XIV. Stigmella, Stigmina, Camptomeris, Polythrincium, and Fusicladiella. Commonwealth Mycol. Inst. Mycol. Papers, No. 49: 1–25. December, 1952. Pages 14–19 and figures 9–13 are devoted to this genus. Hughes recognizes as valid five species: *C. calliandrae, C. leucaenae, C. albizziae, C. desmanthi* and *C. sp.* He includes in *C. albizziae* what is described in this paper as *C. verruculosa* and as *C. sp.* what the writer names *C. martynii.* Hughes did not have available for study material of what the writer names *C. floridana* and *C. albizziicola* and so these are not mentioned. The writer considers that Hughes is in error in including *C. verruculosa* under *C. albizziae.* In his illustrations Hughes shows conidiophores with numerous scars of conidial attachment. Only in one case was the writer able to detect these supplementary conidial scars in the material studied by him. Apparently only one conidium is normally produced on each conidiophore.

MICHIGAN STATE COLLEGE,
EAST LANSING, MICHIGAN

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STUDIES IN THE GENUS PLEOSPORA. V[1]

LEWIS E. WEHMEYER

(WITH 18 FIGURES)

In previous papers the writer has discussed the species of *Pleospora* with 3-septate spores (**5**) and the origin of the vulgaris and leptospheroid series from this group (**6**). It is the concern of this and a following paper to trace the relationships of a rather heterogeneous group of species arising from the 5-septate level of the leptospheroid and vulgaris series.

It was previously pointed out (**5**) that *P. vagans* with 5-septate spores has a spore form similar to that of *P. pellita* with 3-septate spores and can be derived from that species by the insertion of a secondary septum in each of the end cells.

Beyond this 5-septate level, the insertion of secondary and tertiary transverse walls in spores of the leptospheroid type has proceeded in an irregular fashion. In spores of this series with five or more septa it is common to find a secondary septation of the vulgaris type in the two central cells. Likewise spores of the vulgaris series, with five septa, may have secondary transverse septa of the leptospheroid type inserted in their end cells. These two types of septation result in a convergence of these two series and, unless the sequence of septation is known, leaves the derivation of such spores in doubt. There are certain characters, however, which aid in placing such spore types.

In spores with a primary leptospheroid septation there is a distinct tendency for an asymmetrical insertion of transverse septa, which is accompanied by an asymmetry of form. This is usually the result of the insertion of secondary or tertiary septa in the lower end of the spore without a corresponding insertion in the upper portion of the spore. For this reason, in the following discussion, the species having spores showing both the vulgaris and lepto-

[1] Papers from the Department of Botany of the University of Michigan, No. 944.

spheroid type of septation are placed in the leptospheroid series if they are asymmetrically septate, and in the vulgaris series if they are symmetrically septate. This, again, is a more or less arbitrary arrangement adopted for clarity of presentation.

In both these series there is a good deal of variation in spore form and this can be used to advantage in judging relationships, for several groups of species stand out in this respect.

The present paper will be restricted to a discussion of the species with leptospheroid or asymmetric septation of the spores.

Inasmuch as nearly all spores above the 5-septate level show some central cells with a vulgaris septation, there is only one group of two species, outside of *P. vagans*, which shows an exclusive leptospheroid and at the same time a symmetrical septation. Both *P. Galii* and *P. Galiorum* (FIGS. 1, 2), of this group, have broad-fusoid, dark red-brown, inequilateral, nonconstricted spores, similar in form to those of the vulgaris series, but having the transverse septa all laid down first and the vertical septa inserted irregularly, and often lacking from some of the cells. The two species differ only in the number of septa and size of the spores. Young spores of *P. Galiorum*, which are normally 7-septate, may have only three or five septa and be about the same size as those of *P. Galii*. Occasionally spores of *P. Galii* are found with only four septa and they then resemble *P. intermedia* in form.

Collections, referred to by number in the text, are listed at the end of the paper.

Pleospora Galii (Romell in Herb.) sp. nov. FIG. 1

Perithecia 300–350 μ diametro, dispersa, depresse globosa, ultime collapsa, erumpentia; basi sparse tomentoso; pariete 10–15 μ crasso, brunneo, parenchymatoso.

Asci numerosi, clavati, 78–90 μ longi, 8.5–11 μ crassi, crasse membranacei, basi unguiculiformes.

Sporae biseriatae, 16–17.5 μ longae, 6 μ crassae, fusiformes vel clavati-ellipsoidales, 5-septatae, brunneae vel atro-brunneae, paululo inaequilaterales vel curvatae, saepe sursum rotundatae, deorsum angustatae, ad septa haud constrictae; cellulis centralis cum septis verticalibus praedita.

Specimen typicum (n. *169*) vide infra enumeratione locorum. In caulibus *Galii veri*, par Huddinge: Huddinge.

Perithecia 300–350 μ in diameter, scattered, flattened-spheric, finally somewhat collapsed, erumpent, slightly tomentose about the base; wall 10–15 μ thick, of small-celled, brown parenchyma.

Asci numerous, clavate, wall slightly thickened, base somewhat tapered and claw-like, 78–90 × 8.5–11 μ.

Spores biseriate, fusoid- or clavate-ellipsoid, 5-septate, dark yellow to red-brown, usually somewhat inequilateral or curved, commonly rounded at one end and more tapered toward the other, without constrictions, vertical walls usually found in one, or several, central cells, 16–17.5 × 6 μ.

Collections: *160* (type), on *Galium*.

These spores and those of the following species might easily be mistaken for members of the vulgaris series. They are distinctive in their inequilateral and non-constricted form. The septation, however, seems to be of the leptosphaeroid type, for the secondary septa are inserted in the end cells, and many spores can be seen with five transverse but no vertical septa.

Pleospora Galiorum sp. nov. FIG. 2

Perithecia 250–300 μ diametro, 150–200 μ alta, dispersa, in cortice vel ligno immersa, ut maculae pustulatae, atrae erumpentia; pariete 10–150 μ crasso.

Asci numerosi, clavati, 65–76 μ longi, 14–15 μ crassi, crasse membranacei, inter pseudoparaphyses immersi.

Sporae biseriatae, fusiformi-ellipsoidales, 17–21.5 μ longae, 7–8.5 μ crassae, 7-septatae, saepe sursum rotundatae, deorsum angustatae, ad septa non constrictae, septis verticalibus in cellulis quibusquibus vel omnibus, rarius in cellulis terminalibus.

Specimen typicum (n. *334*) vide infra in enumeratione locorum. In caulibus *Galii Molluginis*, ex Suecia.

Perithecia 250–300 × 150–200 μ, appearing on the surface as scattered, hemispheric, swollen, blackened spots beneath which they are immersed within the wood fibers or the bark; walls thick (up to 150 μ), black, composed of a mixture of fungous tissue and blackened host tissue.

Asci numerous, clavate, wall not much thickened, 65–76 × 14–15 μ, imbedded in an abundant interthecial tissue.

Spores biseriate, fusoid-ellipsoid, mostly 7-septate, dark yellow-brown, straight, or mostly somewhat inequilateral or curved, often rounded at one end and tapered toward the other, without constrictions or very slightly constricted at the central septum, vertical septa in one or all of the central cells, occasionally in the end cells, 17–21.5 × 7–8.5 μ.

Collection: *334* (type), on *Galium*, from Sweden.

In spore form this collection is identical with that of *P. Galii*, but an additional septum has been inserted in each end cell. The perithecia however, are somewhat flattened, strongly erumpent and with a perithecial wall that is almost colorless below, in which respect it approaches a *Teichospora* or even a *Hysterographium*.

There is a second collection (*776*), in the Riksmuseum, on *Barbaraea*, which has spores (19.5–23 × 7–9 μ) almost identical to those of *P. Galiorum*, but with more elongate (800–1000 × 300–400 μ) hysteriaceous fruiting bodies, again with a faint indefinite lower well. More collections are needed to determine whether these two collections represent the same species and if so whether it should be considered a *Hysterographium* or a *Pleospora*.

The two following species are unique in the cylindric form of the spore, with one cell enlarged.

PLEOSPORA OBLONGATA Niessl, Not. Pyr. (Verhandl. nat. Ver. Brünn **14**: 177). 1876. FIG. 5

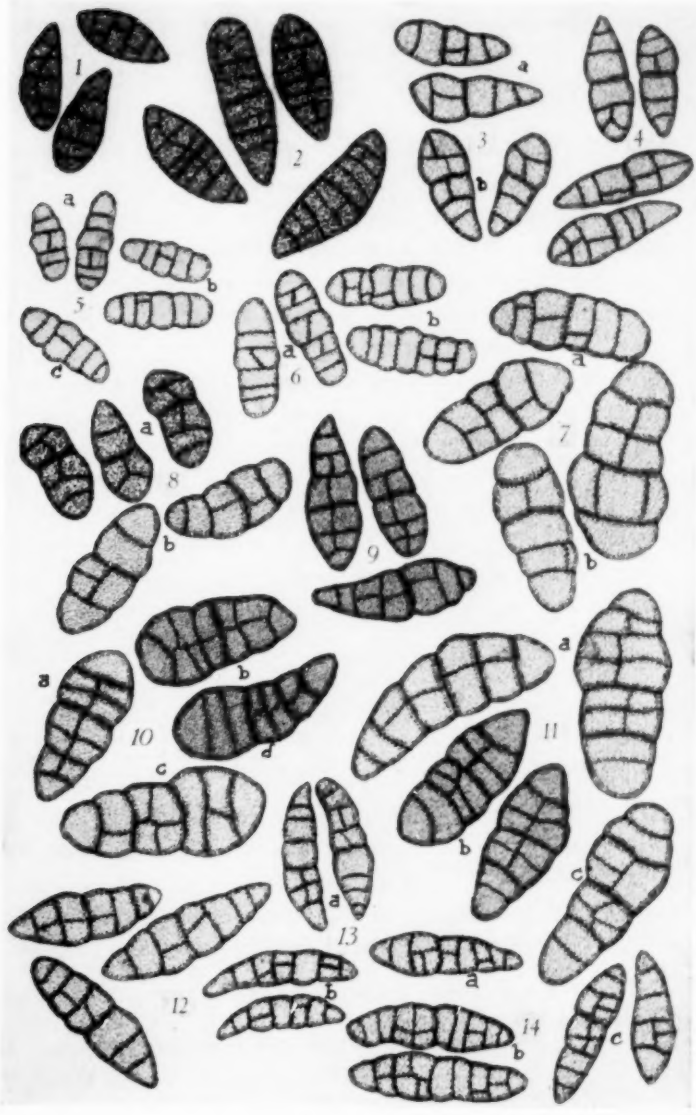
Pleospora oblongispora Rehm, Ann. Myc. **5**: 542. 1907.

Perithecia 150–250 μ in diameter, globose or somewhat flattened, thickly scattered, remaining immersed, erumpent as a short stout ostiolar neck; walls 10–20 μ thick, parenchymatic, smooth.

Asci clavate to cylindric-clavate, wall somewhat thickened, base claw-like, 65–90 × 9–13 μ.

Spores biseriate to uniseriate, oblong, cylindric, yellow-brown to dark yellow-brown, 4–5-septate, ends broadly rounded, not constricted or very slightly constricted at the septa, 5th septum, when

FIGS. 1–14. Ascospores of *Pleospora Galii* sp. nov., from type collection (*169*). 2. *Pleospora Galiorum* sp. nov., from the type collection (*334*). 3. *Pleospora kansensis* E. & E., from collection No. *42* (a) and No. *426* (b). 4. *Pleospora clavispora* sp. nov., from the type collection, No. *337*. 5. *Pleospora oblongata* Niessl, from collections No. *36* (a), *37* (b) and *38* (c). 6. *Pleospora cylindrospora* sp. nov., from the type collection, No. *39* (b) and collection No. *40* (a). 7. *Pleospora Clementsii* sp. nov., from the type collection, No. *43* (b) and from collection No. *82* (a). 8. *Pleospora dichromatricha* (Speg.) Wehm. var. *dichromatricha*, from the type collection, No. *427* (b) and var. *nigriseda*, from collection No. *41* (a). 9. *Pleospora intermedia* Speg., from the type collection, No. *44*. 10. *Pleospora orbicularis* Auersw., from collections No. *46* (a), No. *45* (b), No. *498* (c) and No. *48* (d). 11. *Pleospora trevoicola* Speg., from the type collection, No. *490* (b) and collections No. *347a* (a) and *501* (c). 12. *Pleospora Ilicis* sp. nov., from the type collection, No. *33*. 13. *Pleospora coronata* Niessl, from collections No. *120* (a) and No. *421* (b). 14. *Pleospora dura* Niessl, from the co-type collection, No. *161* (a) and collections No. *121* (b) and No. *122* (c).



FIGS. 1–14.

present, inserted in the upper half of the spore, 3rd or 4th cell somewhat enlarged, vertical walls in one or several of the central, or sometimes the end cells, 12.5–18 × 4.5–6 μ.

Collections: *36, 37, 38,* on *Cirsium, Galium* and *Larix,* from central Europe.

The spores of this species are easily distinguished by their oblong form; they may be either 4- or 5-septate, with the insertion of the extra (5th) septum in the upper half of the spore to give a symmetric septation. If this is the interpretation, the swollen cell occurs below the primary septum and is centrally located in an asymmetrically (**4**) septate spore, which is unusual (see discussion of *L. obtusispora* under next species).

Exsiccati of this species show mixtures of this and the next species as well as other Pleosporas. Niessl, however, gives the spores of his *P. oblongata* as "5 (rarius 4)" septate, and Berlese's figures (**2**: *Pl. 11, fig. 1*), taken from Niessl's material, are typical of the species here described with 4–5 septa. Berlese's placement of *Pyrenophora Sedi* Roum. & Brun. as a synonym of *P. oblongata* is incorrect, however. The type of *P. Sedi* (*57*) shows a *Pleospora* near *P. vulgaris,* and the description states that it is near *P. chrysospora* (Niessl) Sacc., which would indicate a similar spore form.

The type collection of *P. oblongispora* Rehm (*38*) (FIG. 5c), on *Larix,* shows the same 4- to 5-septate spores. Rehm describes them as 5-septate and 15–18 × 6–7 μ. The perithecia occur on the leaf bases of small young twigs.

Pleospora cylindrospora sp. nov. FIG. 6

Perithecia 200–250 μ diametro, globosa vel modice depressa, dispersa, immersa; ostiolo papillaeformi, erumpente; pariete parenchymatoso, 10–20 μ crasso.

Asci clavati vel longe clavati, 85–110 μ longi, 12.5–14 μ crassi, modice crasse membranosi, basi unguiculiformes.

Sporae biseriatae vel uniseriatae, oblonge cylindricae, 18–21 μ longae, 5.5–7 μ crassae, lutei-brunneae, 5- vel plerumque 6-septatae, utrinque late rotundatae, ad septa non constrictae, cellula quarta inflata, septis verticalibus in cellulis centralibus, interdum in cellulis terminalibus.

Specimen typicum (n. *39*) vide infra in enumeratione locorum. In caulibus herbacearum.

Perithecia 200–250 μ in diameter, globose or slightly flattened, thickly scattered, long immersed, erumpent as a small papillate ostiole; walls parenchymatic, 10–20 μ thick.

Asci clavate to clavate-cylindric, walls somewhat thickened, base claw-like, 85–110 × 12.5–14 μ.

Spores biseriate to uniseriate, oblong, cylindric, yellow-brown to dark yellow-brown, 5- or mostly 6-septate, ends broadly rounded, not constricted at the septa, fourth cell somewhat swollen or enlarged, vertical walls occurring in one or several of the central or, sometimes, the end cells, 18–21 × 5.5–7 μ.

Collections: *39* (type) *40*, on herbaceous stems, Central Europe.

This species is obviously derived from the last, by the insertion of an extra (6th) septum in the lower half of the spore. The spores are slightly larger than in *P. oblongata*, but the less common 5-septate ones have the same form, with the swollen cell below the central septum. The insertion of the sixth septum again places this swollen cell in a symmetric position, with two septa on either side of it. No. *39* is selected as the type because it is the earlier collection. On No. *40*, there is an almost illegible notation "var. . . . septata," so that Rehm also noted the difference in septation.

It is possible, of course, that further collections will show intermediates between this and *P. oblongata*, but all collections so far seen have been quite distinct. *Leptosphaeria obtusispora* Speg., on *Yucca gloriosa*, from Argentina, is interesting in this connection. Berlese (**2**: 9) states that the spores of this species recall those of *P. oblongata*. The type collection (La Plata Mus. No. *2393*) shows spores of this same general form, 16–21.5 × 4–5 μ. They are rather narrow, but cover the range in length of both the species just described. They are mostly 5-, rarely 4-septate, but show no vertical septa in any of the spores. The swollen cell in this species, however, lies above the central septum and is the third cell in a 5-septate spore.

The remainder of the leptospheroid series show an asymmetric septation and ascospores which are fusoid, ellipsoid or clavate. In the first group, with 4-septate ascospores, *Pleospora kansensis* (FIG. 3) has spores which are easily derived from the 3-septate spores of *P. pellita* by the insertion of an extra septum in the lower end of the spore. The collection (*23*) of *P. parvula*, pre-

viously discussed (**5**) under *P. vagans*, has this same type of spore, except that in *P. parvula* most of the spores also have an extra septum in the upper half of the spore, making them 5-septate.

P. clavispora (FIG. 4) represents a woody form of *P. kansensis* (FIG. 3). *P. dichromatricha* (FIG. 8), and *P. Clementsii* (FIG. 7), which differ only in the size of their spores, both differ from *P. kansensis* in the darker brown spores with more broadly rounded ends.

The next group which has, typically, six septa in its spores, is apparently derived from a spore type with five septa, by the insertion of an additional septum in the lower end of the spore. This tendency to form additional septa in the lower end appears to be, again, the result of the space relationships in spores which tend to be clavate, with a broad rounded upper portion which is shorter,

TABLE I

Coll. No.	Host	Spores	Asci	Perithecia
		Pleospora oblongata		
36	Galium	12.5–15 × 4–5.5	62–70 × 9–11	200–250
37	Cirsium	14–16 × 5.5–6	70–90 × 10–12	150–250
38	Larix	14–18 × 5–6	62–85 × 11–13	150–200
		Pleospora cylindrophora		
39	Stems	18–21.5 × 6–7	85–100 × 12.5–14	200–250
40	Stems	19–21.5 × 5.5–6	90–110 × 10–13	200–250
		Pleospora kansensis		
426	Azorella	16–19.5 × 7	53–62 × 11–14	200
42	Melilotus	18–21.5 × 7–8.5	55–65 × 14–18	150–250
		Pleospora dichromatricha		
41	Juncus	16–21.5 × 7–9	60–78 × 19–21.5	150–175
427	Euphorbia	19.5–21.5 (28) × 8.5–9	70–85 × 10–11	180–250
		Pleospora Clementsii		
82	Mentha	23–26 × 9–12	75–90 × 21–22	200–300
43	Touteria	26–32 × 12–14	85–90 × 23–25	200–300

TABLE I—*Continued*

Coll. No.	Host	Spores	Asci	Perithecia
		Pleospora orbicularis		
45	Allium	21.5–26.5 × 12–13	85–100 × 21–26	150–300
46	Berberis	23–28 × 10–11	85–90 × 21–26	200–250
47	Berberis	26–30 × 10–12.5	75–85 × 26–28	300–400
48	Berberis	26–32 × 10–12.5	85–90 × 16–18	200–300
49	Berberis	26–33.5 × 11–13	85–90 × 23–27	300–350
503	Colutea	30–35 × 12–14	90–125 × 25–27	150–250
498	Halimodendron	30–36 × 14–16	105–125 × 35	300–400
		Pleospora coronata		
120	Salsola	19.5–25 × 4.5–6	70–78 × 8.5–9.5	200–300
421	Carduus	19.5–26.5 × 5.5–6.5	62–78 × 12.5	200–400
		Pleospora dura		
121	Melilotus	20–25 × 7	74–90 × 12.5	250–350
122	Linaria	23–25 × 7–8	75–90 × 12.5–14	250–300
161	Melilotus	23–30 × 7–9	70–90 × 14–16	300–350
		Pleospora abscondita		
350	Phragmites	28–32 × 7–9	140–160 × 19–23	500x250
162	Aconitum	32–41 × 7.5–10	80–90 × 14	400–500
543a	Silene	37–48 × 9–11		
		Pleospora trevoicola		
490	Trevoa	19.5–25 × 9.5–11	70–78 × 17–19	200–300
501	Genista	25.5–28.5 × 9–10.5	88–97 × 21–23	200–250
347a	Arenaria	33–37 × 11.5–13	125–140 × 26–30	100–150

and a tapered lower portion which is longer and so allows additional septa to be formed therein, below the primary septum. In *P. intermedia* (FIG. 9) the spores are rather narrow and fusoid-clavate, whereas in *P. orbicularis* (FIG. 10) the spores are broader and more definitely clavate. It should be noted that this same tendency in the herbarum series results in asymmetrically septate spores, beyond the 7-septate level (**6**). In the collections placed in *P. trevoicola* (FIG. 11), there is a greater tendency for an additional septum to occur in the upper portion of the spore, also resulting in 7-septate spores. As a result, the variations in spore form

and septation of this species bridge the gap between a number of species in the leptospheroid, vulgaris and herbarum series, with 5- to 7-septate spores.

PLEOSPORA KANSENSIS E. & E. Journ. Myc. **8**: 17. 1902. FIG. 3

Pyrenophora antarctica Speg. Fung. Pat. (Bol. Acad. Cien. d. Cordoba **11**: 51). 1887.

Pleospora antarctica (Speg.) Sacc. Syll. Fung. **9**: 877. 1891.

Perithecia 150–200 μ in diameter, rather widely scattered, globose to depressed or collapsing; with a penicillate fascicle of hyphae 35–50 μ in length about the ostiole in some cases, wall membranous, 10 μ thick, parenchymatic.

Asci rather stout clavate, apical wall somewhat thickened, base claw-like, 53–65 × 11–18 μ.

Spores biseriate to overlapping uniseriate, fusoid-ellipsoid to clavate-fusoid, 3- to 4- (rarely 5-) septate, yellow-brown to dark yellow-brown, often asymmetric, broader and rounded above, tapered below, mostly inequilateral, constricted at the central septum but only slightly so at the other septa, usually with one septum above and two below the central primary septum, vertical septa in one or more of the central cells, rarely in the end cell, rarely with a vulgaris-type septation in the upper central cell, 16–21 × 7–8.5 μ.

Collections: *42* (type), *426*: On *Melilotus* and *Azorella*, from Kansas and Patagonia.

The type of *P. kansensis* (*42*) (FIG. 3a) includes good material with perithecia immersed in stems of *Melilotus*. The type of *P. antarctica* (*426*) (FIG. 3B) contains two fungi on leaves of *Azorella*. One of these has small perithecia with dark brown stiff setae, but the spores in these perithecia are all dark red-brown, almost opaque, collapsed, one- or two-celled and obviously abnormal. A few perithecia with penicillate ostioles were found which contained yellow-brown spores very similar to those described above for *P. kansensis* and agreeing with the drawings on the packet and Spegazzini's description of *Pyrenophora antarctica*. Spegazzini described both a *Pleospora* and a *Pyrenophora antarctica* but Saccardo listed them both under *Pleospora* in his Sylloge (**4**: 877 & 888). The specific epithet of *antarctica* must be retained for Spegazzini's species of *Pleospora*, however, and the epithet *kansensis* is, therefore, used for this species.

Pleospora clavispora sp. nov. FIG. 4

Perithecia 400–500 μ diametro, 250–300 μ alta, globosa, dense dispersa, in cortice immersa, erumpentia; ostiolo papilliformi; pariete 20–50 μ crasso, parenchymatoso.

Asci longe cylindrici-clavati, 85–110 μ longi, 12.5 μ crassi, crasse membranacei, basi unguiculiformes.

Sporae imbricati-uniseriatae, fusiformi-ellipsoidales vel clavatae, 16–21.5 μ longae, 7–8.5 μ crassae, lutei-brunneae, utrinque angustatae vel sursum rotundatae et deorsum angustatae, plerumque cum 1 septo super et 2 septis sub septo primari centrali; septis verticalibus in cellulis centralibus vel interdum in cellulis terminalibus.

Specimen typicum (n. *337*) vide infra in enumeratione locorum. In caulibus *Sorbi Aucupariae*, ex Succica.

Perithecia rather thickly scattered, 400–500 × 250 × 300 μ, flattened spheric, imbedded in the bark fibers, erumpent as small papillate ostioles, walls 20–30 or 50 μ (at the sides) thick, composed of small-celled parenchyma.

Asci long, cylindric-clavate, walls somewhat thickened, base claw-like, 85–110 × 12.5 μ.

Spores overlapping uniseriate, fusoid-ellipsoid to clavate, mostly 4-septate, rarely 3- or 5-septate, light yellow-brown, both ends tapered or, more commonly, the upper end rounded and the lower end tapered, usually with one septum above and two below the central primary septum, vertical septa in the central cells, or sometimes in the end cells, 16–21.5 × 7–8.5 μ.

Collection: *337* (type), on *Sorbus Aucuparia*, from Sweden.

This species is based upon a single undetermined collection in the Riksmuseum Herbarium, at Stockholm. One packet contains perithecia on the inner surface of bark of *Sorbus* and a second packet, from the same locality and the same date, shows perithecia in the bark fibers remaining upon the surface of a reddish discoloration on a decorticated twig. They are both the same fungus and it differs from *P. kansensis* in the larger perithecia, the longer, more cylindric asci and the spores, which are more commonly 3- or 5-septate and have vertical septa in the end cells.

Pleospora dichromatricha (Speg.) comb. nov. FIG. 8

Perithecia 150–250 μ in diameter, either widely or thickly scattered, spheric or flattened, sometimes collapsing, immersed, slightly pustulate, ostiole punctate, wall thin, either smooth or covered over the entire surface with stiff dark brown hairs.

Asci cylindric-clavate to stout clavate, with thickened wall and claw-like base, 60–100 × 10–20 μ.

Spores biseriate, ellipsoid, red-brown, 3- to 5-, mostly 4-septate, with one septum above and two below the central primary septum, mostly straight and somewhat asymmetrical, ends broadly rounded, tapered below the middle, 16–21.5 × 7–9 μ.

Collections: *127* (type) and *41*, on *Euphorbia* and *Juncus*, from the Canary Islands and the Caucasus.

These two collections may prove to be distinct species, but until more and better material can be had for study they are placed here as separate varieties under one species, because of the similarity of their spores.

var. **dichromatricha** stat. nov. FIG. 8b

Pyrenophora dichromatricha Speg. Anal. Mus. Nac. Buenos Aires **26**: 126. 1914.

Perithecia 180–250 μ in diameter, spheric or flattened to somewhat collapsed, covered with a sparse to more abundant tomentum of stiff brown hairs. Asci rather cylindric-clavate, 75–100 × 19–20 μ. Spores 19.5–21.5 μ.

Collection: *127*, on *Euphorbia*, from the Canary Islands.

The type of *Pyrenophora dichromatricha* Speg. has rather agglutinated spores. The spores seen from asci or perithecia measured 19.5–21.5 × 8.5–9 μ. There were also spores scattered about upon the surface of these stems which appeared swollen, were larger (23–28 × 10–12.5 μ), lighter yellow-brown, and commonly showed a fifth septum. These appeared to be exuded spores which were about to germinate. Judging from Spegazzini's description and drawings upon the type packet, these exuded spores were the ones used as a basis for his species.

var. **nigriseda** (Rehm) comb. nov. FIG. 8a

Pleospora infectoria var. *nigriseda* Rehm, Monit. Jard. bot. Tiflis **25**: 13. 1913.

Perithecia 150–175 μ in diameter, scattered on grayish areas of the stem, smooth, ostiole punctate. Asci broad clavate, 60–75 × 19–21.5 μ. Spores 16–21.5 × 7–9 μ.

Collection: *41*, on *Juncus*, from the Caucasus.

The original description of *Pleospora infectoria* var. *nigriseda* Rehm gives the spores as being the same as those of *P. infectoria*, but the type collection has dark red-brown spores which are mostly 4-septate, although the general form is similar to those of *P. infectoria*.

This species differs from the two preceding in the darker red-brown spores which have more broadly rounded ends, and from the next species in the smaller spores.

Pleospora Clementsii sp. nov. FIG. 7

Perithecia 200–300 μ diametro, globosa vel paululo depressa; pariete 10–20 μ crasso.

Asci clavati, 70–90 μ longi, 17–25 μ crassi, crasse membranacei, basi unguiculiformes.

Sporae biseriatae, ellipsoidales vel clavati-ellipsoidales, brunneae, 23–32 μ longae, 10–14 μ crassae, 4-septatae, interdum 5-septatae, ad septa constrictae, utrinque late rotundatae, saepe sursum latae, deorsum angustatae; septis verticalibus in cellulis centralibus, interdum in cellulis terminalibus.

Specimen typicum (n. *43*) vide infra in enumeratione locorum. In caulibus *Touteriae multicaulis*, in republica Coloradensi.

Perithecia scattered, spheric or slightly depressed, 200–300 μ in diameter, wall smooth, 10–20 μ thick.

Asci clavate, thick-walled, base claw-like, 70–90 × 17–25 μ.

Spores biseriate, ellipsoid to clavate-ellipsoid, dark yellow-brown, 4-septate with one septum above and two below the central primary septum or, occasionally, 5-septate, constricted at the septa, vertical septa in the central cells or sometimes in the end cells, ends broadly rounded, often broader above and tapered below, 23–32 × 10–14(–16) μ.

Collections: *43* (type) and *82*, on *Touteria* and *Mentha*, from Colorado.

These two collections, made by Clements in Colorado, are no doubt the same species, and very similar to *P. dichromatrica*, differing chiefly in the large spores. The large spores seen on the surface of the type collection of *P. dichromatica* bridge this gap between the two species. The collection (*82*), on *Mentha*, bears two species of *Pleospora*, one of which is *P. media* and the other this species. Many of the spores of this latter species are 5-septate which gives the impression of *P. vulgaris* or *P. richtophensis* with spores in which one of the septa in the upper half has failed to

form. The collection (*43*) on *Touteria* (FIG. 7b), has somewhat larger spores.

PLEOSPORA INTERMEDIA Speg. Fungi Chilensis 88. 1910. FIG. 9

Perithecia 200–250 μ in diameter, somewhat flattened spheric, scattered, immersed, then erumpent as small papillate ostioles; walls 10–20 μ thick, parenchymatic.

Asci cylindric-clavate, 70(–120) × 10–12 μ, wall thickened, base claw-like, interspersed with interthecial strips.

Spores biseriate, fusoid to clavate, dark yellow-brown to red-brown, mostly 5-septate, but commonly with one or two septa above and two or three septa below the central primary septum, and then 4- or 6-septate (rarely 7-septate), mostly straight, or slightly inequilateral, asymmetric, commonly rounded above and tapered below, slightly or not at all constricted at the septa, usually with a vertical wall in one or two of the central cells, 19.5–26.5 × 7–8 μ.

Collection: *41* (type) on *Asteriscium*, from Chile.

The spores of this species are often 5-septate, but, probably because of the asymmetric clavate form, they tend to have an extra septum in the lower end, differing in these respects from *P. vagans*. In form the spores resemble those of *P. clavispora*, on *Sorbus* (FIG. 4), which have fewer septa, and those of *P. Ilicis* (FIG. 12), which differ in being fusoid rather than clavate.

PLEOSPORA ORBICULARIS Auersw. Hedw. **7**: 184 and Oesterr. bot. Zeitschr. **9**: 274. 1868. FIG. 10

Pleospora Berberidis Rab. in Fung. Eur. 1727.

Pleospora coluteicola Frag. Fung. Hort. Bot. Matrit. 15. (Trab. Mus. Nac. Cienc. Nat., ser. Bot. **12**.) 1917.

Pleospora herbarum forma *Halimodendri* Frag. Fung. Hort. Bot. Matrit. 18. (Trab. Mus. Nac. Cienc. Nat., ser. Bot. **12**.) 1917.

Perithecia 150–400 μ in diameter, flattened-spheric, immersed beneath the periderm which is often blackened as small spots about the ostiole; wall thin, parenchymatous, sometimes with a slight soft tomentum.

Asci stout-clavate, rather thick-walled and with a claw-like base, 80–125 × 21–35 μ.

Spores biseriate, ellipsoid to mostly clavate, mostly 6-septate, with two septa above and three below the central primary septum, yellow-brown to red-brown, usually asymmetric, broader and rounded above, narrower and tapered below, straight to inequilat-

eral or curved, constricted at the central primary septum, slightly or not at all at the other septa, with vulgaris type vertical septa in the two central primary cells and vertical septa commonly in the upper end cell and lower penultimate cell, 21–36 × 10–16 μ. Some spores are found with four or five septa. These are more abundant in some collections than others.

Collections: *45, 46, 47, 48, 49, 498, 503*, on *Berberis, Allium, Colutea* and *Halimodendron*.

This species characteristically occurs on *Berberis*, but collections on other hosts have been found which appear to be the same species. Winter (**7**) states that he compared the type of *P. orbicularis* with Fung. Eur. N. *1722*, of *P. Berberidis* and found them to be the same. Berlese (**1**) repeats Winter's description, but later (**2**), on the basis of a Celloti specimen, he gives the spores as 20–22 × 7–8 μ and figures (**2**: *Pl. 17, fig. 2*) 5- to 6-septate spores of the *P. vagans* or *P. dura* type, which is incorrect. Collection No. *45* is given as *Pleospora oligotricha* and as on *Allium*, but it is this species with rather small spores (FIG. 10b). The type (*503*) collection of *Pleospora coluteicola* Frag., first sent to the writer by Dr. Urries, showed nothing but the *Phoma* reported by Fragoso as associated with this species. A second sending yielded a *Pleospora* belonging to this species. Fragoso's original description, however, gives the spores as hyaline at first, then olive-brown, 3-septate and 18–22 × 9–11 μ. Either Fragoso had very immature material of this species or some other species of *Pleospora* no longer present on the type. The name is placed here doubtfully as a synonym. The type collection of *P. herbarum* forma *Halimodendri* Frag. (*498*) has the same clavate 6-septate spores (FIG. 10c), but more of them show seven septa, in which condition they resemble *P. herbarum*. These last two collections also have somewhat larger spores (30–36 × 12–16 μ).

PLEOSPORA TREVOICOLA Speg. Fung. Chilenses 92. 1910. FIG. 11

Pleospora herbarum forma *Genistae* Frag. Fung. Hort. Bot. Matrit. 18. (Trab. Mus. Nac. Cienc. Nat., ser. Bot. **12**.) 1917.

Perithecia 100–300 μ in diameter, somewhat flattened-spheric, immersed or erumpent-superficial, on surface as circular blackened spots, with a light colored central area in some cases.

Asci clavate, base claw-like, wall thickened, 60–97(–140) × 17–23(–30) μ.

Spores biseriate, fusoid, oblong ellipsoid or clavate, 5- to 7-septate, yellow-brown to red-brown, straight to slightly inequilateral or curved, symmetric or asymmetric with a broader shorter upper portion, ends rounded or bluntly tapered, constricted at the central septum, the two central cells usually with a vulgaris-type septum, commonly a third (secondary) septum in the lower end, sometimes also in the upper half, vertical septa occasionally in the end cells, 19.5–37 × 7–13 μ.

Collections: *347a, 490* (type), *501,* on *Arenaria, Genista* and *Trevoa.*

These three collections are placed under this species somewhat arbitrarily because they all show the same type of extremely variable spores. This condition is difficult to interpret. It suggests a heterokaryotic condition of the mycelium, for the spores appear to bridge the gap between *P. orbicularis* in the asymmetric series and *P. punctata* or *P. tomentosa* in the symmetric series.

Each of the three collections shows three general kinds of ascospores, usually within the same perithecium. Some spores are symmetric and 5-septate, much as those of *P. richtophensis* (FIG. 11b). Others are either fusoid or clavate with a broad rounded upper portion and have an additional septum in the lower end and resemble the spores of *P. orbicularis* (FIG. 11c). Still others are fusoid, 7-septate (with an extra septum in each end) (FIG. 11a) and resemble the spores of *P. punctata,* etc. Certain collections of *P. media* var. *acuta* (i.e., No. *321*) are very similar to these collections, differing only in the presence of vertical walls in the end cells of a greater number of spores.

The spores of the three collections vary a great deal in size, but this range is about the same as that of *P. orbicularis.* The type collection of *P. trevoicola* is on disintegrated bark covered largely by pycnidia of a *Sphaeropsis* and a *Diplodia,* and Spegazzini's perithecial measurements of 150–180 μ must be confused with these fruit bodies. The spores in the few perithecia seen were old, collapsed and agglutinated. They were small (19.5–25 × 9.5–11 μ) and dark red-brown. The type (*501*) of *P. herbarum* f. *Genistae* has somewhat larger (24–28 × 9–10 μ) spores (FIG. 11c) which are yellow-brown in color. Collection No. *347a* (FIG. 11a) has

much larger spores (33–37 × 11–13 μ) but shows the same variation in form and septation.

In the following group of species, which have ascospores that are more acutely tapered, there is a great deal of variability in spore septation and it is often difficult to circumscribe species. This is due to the fact that as the spore increases in size with maturity, extra septa are laid down in both ends of the spore, often in an irregular fashion. A further complication is the occurrence of vulgaris septation in the central cells of these spores. As a result, a given collection may show spores with varying septation, some simulating species of the asymmetric series with fewer septa, and others simulating corresponding ones of the symmetric series with additional septa. It is necessary, in each collection, to consider the most mature spores as well as the commonest type of septation and the range of variation.

There are a number of such collections which fall within the range of Niessl's three species, *P. coronata*, *P. dura* and *P. setigera*. The spores of these collections vary in the number of their septa from four to nine, often within a single collection. The descriptions in the literature reflect this situation, and it is next to impossible to fix these species names without type material, which, it seems, Niessl did not leave us. The tabulation, below, of the characters given for these species by Niessl and Berlese, from an examination of Niessl's material, illustrates this situation.

	Perithecia	*Asci*	*Spores*
P. coronata			
by Niessl	fibrillose & penicillate	60–100 × 13–18	22–27 × 7–9 (6- to 8-septate)
by Berlese		120–150 × 15–17	24–30 × 6–9 (6-septate)
P. dura			
by Niessl	fibrillose	120–150 × 15–17	24–30 × 8–9 (7- to 9-septate)
		100–120 × 12–14	32–34 × 8–9 (7- to 8- or 9-septate)
P. setigera			
by Niessl	fibrillose & setose	90–120 × 14–15	22–30 × 8–10 (4- to 5-septate)
by Berlese		70–80 × 8–9	20–24 × 8–9 (4- to 5-septate)

Niessl used the penicillate ostiole to distinguish *P. coronata*. It is doubtful whether this is a constant character. It was seen only

in two collections of this series, which were No. *44*, of *Pleospora coronata*, and the type of *Pyrenophora antarctica* (No. *426*), and seems to be a character of young perithecia rather than any one species. The epithet *coronata* is used, rather arbitrarily, here for a group of collections with narrow, curved ascospores which are mostly 6-septate and do not show any of the vulgaris type of septation in their central cells, whereas *P. dura* is used for those collections with larger, straighter spore types with variable septation (4 to 9 septa) and often with the vulgaris type of septation in the central cells. *P. Ilicis* is a species on woody substrata which has broader spores than *P. coronata* and they are more commonly 5-septate. It represents a variation of *P. vagans*, with an extra septum in the lower end of the spore.

Pleospora Ilicis sp. nov. FIG. 12

Perithecia 300–500 μ diametro, globosa, pustulata, erumpentia; ostiolo papillato; pariete 50–70 μ crasso, intus hyalino, extus atri-brunneo.

Asci clavati, 85–110 μ longi, 12.5–14 μ crassi, paululo crasse membrancei.

Sporae biseriatae vel imbricati-biseriatae, fusiformes, 23–26 μ longae, 7–8 μ crassae, 5- vel 6-septatae, rectae vel modice inequilaterales, plerumque symmetricae, vel infra angustatae, ad septa constrictae, cum septis verticalibus in cellulis centralibus, non in cellulis terminalibus.

Specimen typicum (n. *33*) vide infra in enumeratione locorum. In caulibus *Ilicis paraguaycnsis*, ex republica Argentinensi.

Perithecia 300–500 μ in diameter, globose, appearing on the surface as thickly scattered pustulate swellings, finally rupturing the periderm; ostioles papillate or short conic; walls parenchymatic, 50–70 μ thick with an outer black-brown and an inner hyaline layer.

Asci clavate, wall slightly thickened, 85–110 × 12.5–14 μ.

Spores biseriate to overlapping uniseriate, fusoid, yellow-brown, 5- or 6-septate, with an additional septum in the lower end, mostly straight or very slightly inequilateral, mostly symmetric, or slightly more tapered below, constricted at the central septum, usually with a vertical septum in one or several of the central cells, 23–26(–32) × 7–8 μ.

Collection: *33* (type), on *Ilex*, from Argentina.

There are apparently two syntype collections of *Leptosphaeria Yerbae* Speg., in the La Plata Museum; No. *2377* yielded the *Leptosphaeria* as described, whereas No. *2378* yielded a dothideaceous *Macrophoma* and the above *Pleospora*. The spores are like

those of *P. vagans*, but commonly show an extra (sixth) septum in the lower end. The spores are broader and straighter than those placed under *P. coronata*.

PLEOSPORA CORONATA Niessl, Notiz. Pyr. (Verh. nat. Ver. Brünn **14**: 176.) 1876. FIG. 13

Perithecia 200–400 μ in diameter, globose or somewhat flattened above, sometimes collapsing pezizoid, thickly scattered, immersed then erumpent as small black spots, sometimes tomentose about the base; walls rather thick (10–50 μ), parenchymatic.

Asci clavate with a somewhat tapering base and a claw-like attachment, wall not much thickened, 65–80 × 8.5–12.5 μ, with scattered interthecial elements.

Spores biseriate above, uniseriate below, yellow-brown, to dark yellow-brown, fusoid, 5-septate at first but with an additional septum in either the lower or both end cells, giving a 6- or 7-septate spore, somewhat broader above the middle and longer tapered below, third (or fourth) cell usually somewhat enlarged, vertical walls in one or more central cells, 19.5–26 × 4.5–6.5 μ.

Collections: *120, 121*, on *Carduus* and *Salsola*, from Italy and North Dakota.

These collections are placed in *P. coronata* even though they do not always show a penicillate ostiole. The spores are first 5-septate but may become 6- or 7-septate by the insertion of a tertiary septum in one or both ends. They differ from *P. Ilicis* in being narrower and more inequilateral, and from *P. dura* in being narrower, having fewer septa, in general, lacking any vulgaris-type septations and in having the central cells more commonly swollen. The perithecia in collection No. *120* soon become exposed and superficial and resemble those of *Teichospora*.

PLEOSPORA DURA Niessl, Notiz. Pyr. (Verh. nat. Ver. Brünn **14**: 178.) 1876. FIG. 14

Pleospora Niessleana Kze. in Kze. Fung. sel. exs. *71*. 1875.

Perithecia 250–350 × 150–250 μ, usually somewhat flattened-globose, thickly scattered, appearing on the surface as circular, blackened flat or sunken spots with a minute central erumpent ostiole, smooth or with a few tomentose hyphae; wall 10–20 μ thick, of small-celled parenchyma.

Asci clavate, tapered toward the base and with a claw-like attachment, wall not much thickened, or slightly so at the apex, 70–90 × 12.5–16 μ, interspersed with interthecial strips.

Spores biseriate above, uniseriate below, fusoid, to clavate-fusoid, (4–)5–7(–9)-septate, yellow-brown to dark yellow-brown, mostly straight or slightly inequilateral, mostly asymmetric, more tapered below, constricted at the primary septum, vertical septa in one or several of the central cells, one to three septa above and two to four below the primary central septum, 20–26(–30) × 7–9 μ.

Collection: *121, 122, 161* (co-type): On *Melilotus* and *Linaria*, from Central Europe.

The spores of these collections show a great deal of variation in the number of septa, even within the same collection. Petrak (3: 218) has reported a similar wide range of variation in spore form and septation for this species, but judging from his spore measurements he has included collections which would be placed in other species of this and also the vulgaris series, as here conceived. In No. *122* there are a number of spores (FIG. 14c) with only one septum above and two below the primary central septum (4-septate). Other spores, in this and other collections, have two or three septa in the upper and two, three or sometimes four septa in the lower portion of the spore, resulting in 5-, 8-, or even 9-septate spores. Collection No. *161*, which is issued in Kunze's Fung. sel. exs. No. *71*, as *P. Niessleana*, and is one of Niessl's co-types, shows the larger spores (FIG. 14a) with more numerous septa. The spores of this species often show vulgaris-type secondary septa in the central cells.

The writer has seen three collections with spores having more than nine septa. There are certain differences between these three collections, but the spores of all three have the same general plan of septation. There is so much variation among the spores of any one collection that they should be united, at least until more material can be studied.

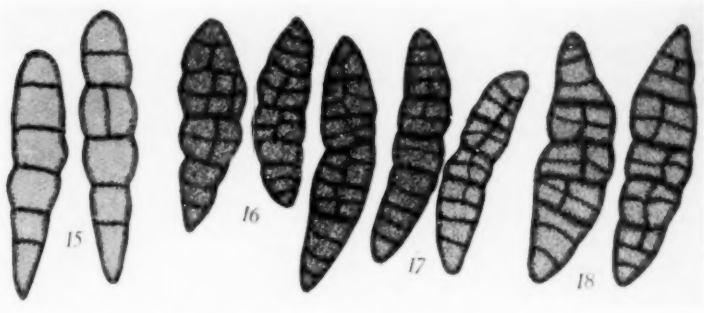
PLEOSPORA ABSCONDITA Sacc. & Roum. in Rel. Libert., ser. 2, No. *157*. (Rev. myc. **1881**: 46.) FIG. 16

Perithecia scattered on purplish or blackened areas of the stem, flattened-spheric, 400–500 × 250–300 μ, causing small pustules on the surface; wall rather thin, 10–30 μ thick, of small-celled parenchyma.

Asci long-clavate with a somewhat tapered base and a claw-like attachment; wall somewhat thickened, 90–160 × 15–23 μ.

Spores biseriate, fusoid or clavate-fusoid, yellow-brown to dark yellow-brown, 6–12(–13)-septate, straight or slightly inequilateral or curved, asymmetric, upper end rounded at first, later tapered but broader than the lower more tapered half, two to three septa above and three to four (or five) septa below the primary septum, and later with vulgaris-type septa in two or four central cells, one, rarely two vertical septa in several central cells, not in the end cells, constricted at the central septum, scarcely so at the other septa, 28–48 × 7–11 μ.

Collections: *162, 350, 543a, 601,* on *Phragmites, Aconitum* and *Silene,* from Switzerland, Tyrol and Washington.



FIGS. 15–18. Ascospores of *Pleospora abscondita* Sacc. & Roum. 15. Spores from collection No. *601.* 16. From collection *350.* 17. From collection No. *162.* 18. From collection No. *543a.*

The use of this binomial for these collections is open to question. The original description gives the spores as 7-septate, but Berlese (**2**: *Pl. 19, fig. 1*) figures spores, taken from Saccardo's original collection, as 7- to 9-septate and with vulgaris-type septa in the central cells. Roumeguère's Fung. Gall. No. *1598,* in the Farlow Herbarium, shows only a *Leptosphaeria* with 3-septate spores. Rehm's Asc. No. *1140,* of *P. abscondita,* in the Farlow Herbarium (*601*), yielded spores (FIG. 15) which were only 5-septate and showed few vertical septa. This may be a distinct *Leptosphaeria,* but it has the same large perithecia as *P. abscondita* and may be an immature condition of that species. This same exsiccatus, in the Riksmuseet, yielded a few spores with five to seven septa, but

most of them showed nine or more septa. Collection No. *543a* (FIG. 18) consisted only of numerous spores found on the surface of stems from which only *P. comata* perithecia were taken. These scattered spores were 10–12- (or 14-) septate.

The species as represented by these collections seems to have immature spores which are 5-septate and without vertical septa; in more mature spores there are basically two or three septa above and three or four, or rarely five, below the central primary septum. In addition, secondary septa of the vulgaris-type are laid down in two or four of the central cells in fully mature spores.

Collections *350* (FIG. 16) and *543a* (FIG. 18) show the same general plan of septation but the spores of *543a* are somewhat larger. Collection No. *162* has spores (FIG. 17) which are more commonly found with a symmetric septation than in the other two collections (i.e., with four or five septa on each side of the central one).

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33. *Leptosphaeria Yerbae* Speg., on *Ilex paraguayensis*, Missiones, San Pedro, Argentina, Feb. 1907 (La Plata Mus. *2378*) (Type).
37. *Pleospora oblongata* Niessl, on *Cirsium*, Straza bei Velder, July 1891 (Riksmuseet: ex Herb. Rehm).
36. *Pleospora oblongata* Niessl, on *Galium Mollugo*, July 1875 (Riksmuseet: ex Herb Rehm).
38. *Pleospora oblongispora* Rehm, on *Larix decidua*, Mieringen, Schweiz, Aug. 7, 1905, leg. O. Jaap. (Riksmuseet: Herb. Rehm) (Type).
39. *Pleospora oblongata* Niessl, on herbaceous stems, Sulden Gletscher im Ortler, July 1889, leg. Rehm (Riksmuseet: Rehm Herb.).
40. *Pleospora oblongata* Niessl, on herbaceous stem, May 1900, leg. Rehm (Riksmuseet: ex Herb. Rehm).
41. *Pleospora infectoria* var. *nigriseda* Rehm, on *Juncus Gerardi*, in monte Kalmas, Prov. Baku, distr. Dzevat, May 1912, leg. Woronin (Riksmuseet: ex Herb. Rehm *297a*) (Type of var.).
42. *Pleospora kansensis* E. & E., on *Melilotus alba*, Rooks Co., Kans., March 12, 1902, leg. E. Bartholomew (N.Y.B.G.: Fungi Col. *1936*) (Type).
43. *Pleospora herbarum* (Fr.) Rab., on *Touteria multicaulis*, Sulphur Springs, Colo., July 23, 1907 (Farl. Herb., Clem. Cr. Form. Colo. *442*).
44. *Pleospora intermedia* Speg., on *Astericium chilense*, Concepcion, Chile, Jan. 1909 (La Plata Mus. No. *2169*) (Type).

45. *Pleospora oligotricha* Niessl, on *Allium* sp., bei Insel, June 4, 1942, leg. K. H. (Richmeyer) (Riksmuseet: ex Herb. Mus. H. Nat. Vindob.).

46. *Pleospora Berberidis* Kze., on *Berberis vulgaris*, Rollsdorf (Sachsen), June, leg. J. Kunze, Winter No. *3750* (Riksmuseet, Rab. Fung. Eur. *1722*) (Type).

47. *Pleospora orbicularis* Awd., on *Berberis vulgaris*, Leutschau (Zips) Ungarn, Aug. 1888, leg. Bresadola (Riksmuseet: ex Herb. Bresadola *259*).

48. *Pleospora Berberidis* Kze., on *Berberis*, Tyrol, Aug. 1872, leg. Rehm (Riksmuseet: ex Rehm Herb.).

49. *Pleospora orbicularis* Awd., on *Berberis*, Waldrand bei Neufriedenheim, München, March 1902, leg. Rehm (Riksmuseet, Rehm Asc. *1439*).

57. *Pyrenophora Sedi* Roum. & Brun., on *Sedum alba*, Gallia, Saintes, Charents-inferieure, March 1885, leg. P. Brunaud (Riksmuseet: ex Herb. Sydow) (Type).

70. *Pleospora Tragacanthae* Rab., on *Phaca frigida*, Furstenalp, Graubunden, 1800 m., Aug. 15, 1903, leg. A. Volkart (Riksmuseet: Rehm Asc. *1566*).

82. *Pyrenophora phaeocomoides* Sacc., on *Mentha penarda*, Boulder, Colo., July 29, 1907 (Farl. Herb., Clem. Cr. Form. Colo. *452*, pr. p.).

120. *Pleospora Lecanora* (Fabre) Rehm, on *Salsola Tragus*, Kulm, N. Dak., July 19, 1913, leg. J. Breneckle (Riksmuseet: ex Rehm Herb.: ex Breneckle Herb., Fung. Dak. *240*).

121. *Pleospora dura* Niessl, on *Melilotus officinalis*, Mähr-Weisskirchen, Aug. 22, 1922, leg. Petrak (Riksmuseet, Petr. Fl. Boh. & Mor. *1875*).

122. *Pleospora dura* Niessl, on *Linaria genistifolia*, Brunn in Mähren, Aug. 1927, leg. J. Hruby (Riksmuseet, Petr. Fl. Boh. & Mor. *2438*).

160. *Pleospora Galii* Romell inedit., on *Galium verum*, Sdl. par Huddinge: Huddinge, April 29, 1894, leg. Romell (M.A. *3689*) (Riksmuseet: ex Herb. L. Romell *16094*).

161. *Pleospora Niessleana* Kze., on *Melilotus alba*, Oberreisdorfer Thal pr. Islebiam (Saxony), May 1875, leg. J. Kunze (Riksmuseet, Kze. Fung. sel. 71) (Type).

162. *Pleospora dura* Niessl, on *Aconitum*, Kartel, Gletscher, Morsthal (Tyrol), July 1878, leg. Britzelmayer (Riksmuseet: ex Herb. Rehm).

334. (*Pleospora?*), on *Galium Mollugo*, Gottland: Bro, June 18, 1920, leg. T. Vestergren (Riksmuseet: Fung. Suec.).

337. (*Pleospora?*), on *Sorbus Aucuparia* bark, Bromma, Drottningsholmsvagert, Nov. 21, 1920, leg. T. Vestergren (Riksmuseet).

347. (*Pleospora?*), on *Arenaria uintahensis*, Wasatch Mts., Salt Lake City, Utah, June 7, 1904, leg. A. O. Garrett (Riksmuseet: ex Herb. Sydow).

347a. Same data; second fungus.

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421. *Pleospora coronata* Niessl, on *Carduus sphaerocephalus*, Italy: Conegliano, Aug. 1878 (La Plata Mus. No. *7185*).

426. *Pyrenophora antarctica* Speg., on *Azorella trifurcata*, Gregory Bay, Patagonia, June 1882 (La Plata Mus. No. *2198*) (Type?).

427. *Pyrenophora dichromatricha* Speg., on *Euphorbia terracina*, Puerto Luz, Las Palmas, Dec. 1913 (La Plata Mus. *2199*) (Type).

490. *Pleospora trevoicola* Speg., on *Trevoa trinervis*, Chile: Santiago, colinas de San Bernardo (La Plata Mus. No. *2151*) (Type).

498. *Pleospora herbarum* f. *Halimodendri* Frag., on *Halimodendron argenteum*, prope Matritis, in Hort. Bot., Aug. 1916, leg. Caballero (Herb. Jard. Bot. Madrid: Fung. *2101*) (Type).

501. *Pleospora herbarum* f. *Genistae* Frag., on *Genista iberica*, Madrid, in Bot. Gard., Aug. 26, 1916, leg. Caballero (Herb. Jard. Bot. Madrid: Fung. *2121*) (Type of form).

503. *Pleospora coluteicola* Frag., on *Colutea tragacanthoides*, prope Matritis, in Hort. Bot., Aug. 26, 1916 (Herb. Jard. Bot. Madrid: Fung. *2178*) (Type).

543. *Pleospora comata* Niessl, on *Silene Macounii*, Frozen Lake, Mt. Rainier, Wash., 6,700 ft., Aug. 17, 1948, leg. E. G. Simmons No. *1969* (Wehm. Herb.).

543a. Same data, second fungus (*Pleospora abscondita* Sacc. & Roum.).

601. *Pleospora abscondita* Sacc. & Roum., on *Phragmites*, Ufer des Bodensee bei Kreuzlingen (Shweiz), Oct. 1892, leg. Wegelin (Farl. Herb., Rehm Asc. *1140*).

776. Undetermined, on *Barbaraca stricta*, Suecia: Storlien, Jemtlandiae, June *22*, 1932, leg. A. G. Eliasson (Riksmuseet: Fl. Suec.).

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THE CONNECTION BETWEEN BOTRYOTINIA FUCKELIANA AND BOTRYTIS CINEREA [1]

J. Walton Groves [2] and Constance A. Loveland [3]

(with 4 figures)

The successful production of apothecia in culture from isolates of *Botrytis* species of the *cinerea* type was reported by Groves and Drayton (**1939**). The apothecia belong to the genus *Botryotinia* Whetz. in the family Sclerotiniaceae Whetz. (**1945**). Although de Bary (**1866**) claimed that *Botrytis cinerea* and a discomycete, which he named *Peziza fuckeliana*, were different states of the same fungus, no attempt was made by Groves and Drayton to name their fungus because the exact identity of de Bary's *Peziza fuckeliana* was in doubt.

Differences of opinion arose in mycological literature concerning the connection between *Botrytis cinerea* and *Peziza fuckeliana*. The literature on this point has been well reviewed by Buchwald (**1949**). He came to the conclusion that the weight of evidence favoured the view that *Peziza fuckeliana* had a *Botrytis* of the *cinerea* type as its conidial state, but he felt that it was probably retricted to *Vitis* as the host plant. It now seems possible to provide conclusive evidence for the connection between the two states and also that the fungus is not restricted to *Vitis*.

Gregory (**1949**) reported the finding of slides of de Bary's original material of *Peziza fuckeliana* in the British Museum in excellent condition. He was able to make a study of this material and published notes and photographs together with a full discussion of the literature pertaining to the connection between these two states. Dried material of the apothecia produced in our cultures

[1] Contribution No. 1194 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

[2] Senior Mycologist, Central Laboratory, Ottawa.

[3] Assistant Mycologist, Central Laboratory, Ottawa.

was sent to Gregory who very kindly made an examination of it and reported *(in litt.)* that it appeared to be identical with *Peziza fuckeliana*.

In addition to this morphological evidence, certain cultural evidence as to the identity of our material with that of de Bary is now available. *Peziza fuckeliana* was originally described as occurring on *Vitis* in the Rhine valley. Since apothecia of many of the Sclerotiniaceae are very similar morphologically, it was considered necessary to establish that matings were possible between our single ascospore cultures and strains of *Botrytis cinerea* from the type host and as closely as possible from the type locality.

In the original experiment in which the apothecia were first obtained, they were developed in nine isolates originating from apples, potatoes, and celery (FIG. 1). By the use of single ascospore cultures, attempts were then made to determine the type of mating behaviour in each of these nine isolates.

The technique used was essentially the same as that described by Drayton (**1937**) with slight modifications. The usual experimental schedule was as follows:

1. The fungus was planted on Petri dishes of potato dextrose agar and grown at room temperature for two to four days.

2. Freshly growing mycelium was transferred to wheat plates. These were prepared by adding 8 gms. of wheat and 25 cc. of water to each Petri dish and sterilizing in the autoclave. A soft wheat, such as Dawson's Golden Chaff, proved to be the most satisfactory for preparing the wheat plates.

3. After two or three days at room temperature the wheat plates were then placed at 5° C or 14° C in the dark for a period of four to six weeks or until the sclerotia were well developed.

4. The sclerotia were then removed from the wheat plates with a pair of fine, sterile forceps and placed on moist, sterile, quartz sand in 100 mm. preparation dishes. The dishes were then placed in the dark at 0° C for at least one month.

5. The sand cultures were transferred to 5° C in the dark for two weeks.

6. Spermatization was carried out and the sand cultures moved to 14° C for one month.

7. The dishes were placed in the greenhouse exposed to north light and shaded from direct sunlight. The greenhouse temperature was kept at about 10–14° C as closely as possible, and sterile water was added to the dishes from time to time to keep the sand moist.

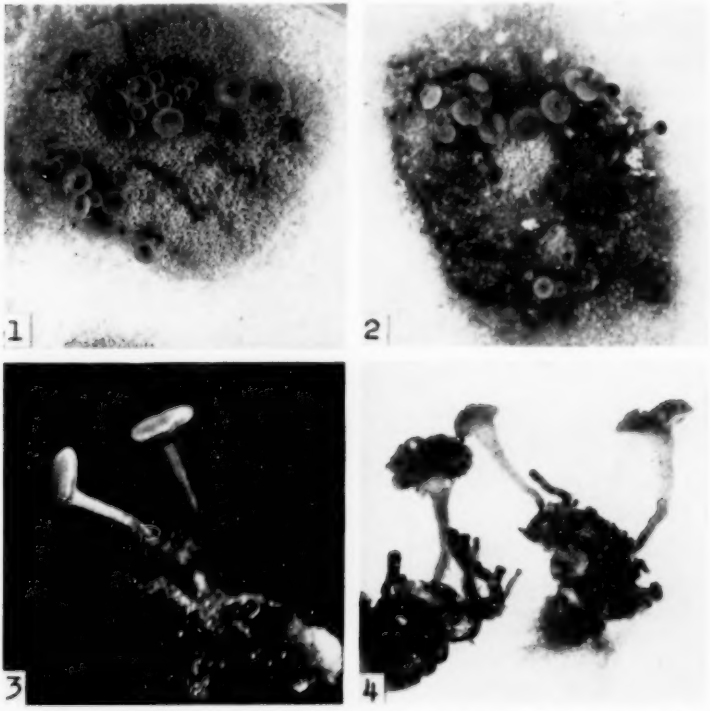

FIG. 1. Apothecia of *Botryotinia fuckeliana* developed in culture, ×1. 2. Apothecia of *B. fuckeliana* from a cross between a single ascospore culture and a Swiss isolate from *Vitis*, ×1. 3. Two apothecia enlarged, ×2 approx. 4. Abnormal multiple head development believed to be related to high temperatures in the greenhouse, ×2.

It was found that better results were obtained when the sclerotia were covered with a thin layer of moist sterilized soil before the dishes were placed in the greenhouse. Sometimes this was done at the same time as the spermatization by adding the spermatial suspension to sterilized soil which was then placed on the group

of sclerotia and distributed over them with a sterilized camel's hair brush. Equally satisfactory results were obtained by painting the suspension on to the sclerotia directly, and adding the soil later before they were moved to the greenhouse.

At first, in an effort to conserve space and glassware, sclerotia from several isolates were spaced some distance apart on the sand in the same dish, but, from the inconclusive results obtained, it was soon realized that it was possible for the spermatia to be washed from one isolate to the other, and that it was absolutely essential to keep each isolate in a separate dish throughout the entire experiment.

TABLE I

CROSSES BETWEEN SINGLE ASCOSPORES OF B56 FROM CELERY

♀ \ ♂	CK	SS2	SS5	SS24
SS1	−	−	+	−
SS2	−	−	+	−
SS5	−	+	−	+
SS9	−	+	−	+
SS20	−	−	+	−
SS21	−	−	+	−
SS22	−	−	+	−
SS23	−	−	+	−

From these experiments it was established that the single ascospore cultures exhibit the bipolar type of mating behaviour similar to that found in *Stromatinia gladioli* (Drayt.) Whetz. by Drayton (**1934**). The results of one experiment with cultures from single ascospores obtained from apothecia of isolate B56, originally isolated from celery in storage, are illustrated in TABLE I.

The appearance of apothecial fundaments was considered to be sufficient evidence that fertilization had occurred, for no fundaments were ever observed in dishes containing unspermatized or self-spermatized sclerotia or as the result of a theoretically infertile cross. Usually the apothecia matured, but in certain in-

stances they failed to complete their development for a number of reasons. An adverse effect was noted as a result of the drying of the sand in the dishes, or when they were subjected to sudden drops or undue rises in temperature. Under these conditions the apothecial fundaments turned black and further development ceased. Another abnormality that was observed, that appeared to be related to high temperatures in the greenhouse, was the production of several smaller discs on one stipe instead of the usual single disc (FIG. 4).

After the initial experiments that led to the development of apothecia, successful crosses were made between single ascospore cultures of the different isolates. The results of such crosses between the isolates B56 and B71, from celery and potato stalks respectively, are given in TABLE II.

TABLE II

CROSSES BETWEEN SINGLE ASCOSPORES OF B56 FROM CELERY AND B71 FROM POTATO

♀ \ ♂	$B_{56}SS_2$	$B_{71}SS_3$	$B_{71}SS_4$	$B_{71}SS_9$
$B_{56}SS_5$	+	+	−	+
$B_{56}SS_8$	+	+	−	+
$B_{71}SS_9$	−	−	+	−
$B_{71}SS_{20}$	−	−	+	−

Single ascospores were isolated from the apothecia developed from these crosses and successful crosses were also made between single ascospores from the other isolates. Hence it was concluded that all these isolates belonged to the same species.

It was then considered necessary to prove that crosses were possible between these single ascospore cultures and isolates of *Botrytis cinerea* from *Vitis* collected in the Rhine valley. Due to the war this work had to be abandoned for some years, but the single ascospore cultures were maintained and in 1947 the senior author had the opportunity to visit Zurich, Switzerland where, through the kind cooperation of Dr. L. Ettlinger and Dr. S.

Blumer, it was possible to obtain two isolates of *B. cinerea* from Malans and Waedenswils, Switzerland. Crossing experiments were then undertaken between these two isolates and our single ascospore cultures.

Since the two isolates from *Vitis* were made directly from nature, it was possible that both mating strains were present and, therefore, if they were used as females, it was possible that any apothecia resulting would not be due to fertilization by the single ascospore culture used as a male. This difficulty was overcome by using the Swiss isolates as males, since any apothecia that developed must result from a fertilization of the single ascospore culture by spermatia from the Swiss isolate.

With the isolates from *Vitis* as males, fertilization was effected in five of the single ascospore cultures and single ascospores were isolated from the apothecia that matured (FIGS. 2, 3). It was, therefore, concluded that the isolates from *Vitis* from Switzerland belonged to the same species as the isolates from apples, potatoes, and celery from Canada.

In 1939, samples of a shipment of grapes var. Emperor shipped from California to Moncton, N. B., were received at the Division of Botany and Plant Pathology. These grapes had arrived in a damaged condition and were attacked by *Botrytis cinerea*. Isolations were made from this material and crossing experiments undertaken with our single ascospore cultures. Apothecia were obtained when the California isolates were used as females, but apothecia also appeared in the unspermatized checks and it is believed that the original isolates were a mixture of strains. Ten single ascospore cultures were isolated, but they were unfortunately lost before further tests could be carried out with them. It seems probable that the California isolates also belonged to the same species as the Canadian and Swiss isolates, but this was not completely established.

However, it has been established that (1) isolates of *B. cinerea* produce sclerotinioid apothecia when compatible strains are crossed, (2) single ascospores from these apothecia give rise to typical fructifications of *B. cinerea*, (3) single ascospore cultures are self-sterile and exhibit bipolar mating reaction, (4) isolates of

B. cinerea from *Vitis* from Switzerland are interfertile with Canadian isolates from apples, potatoes, and celery, (5) apothecia from such crosses are morphologically similar to the type of *Botryotinia fuckeliana* (de Bary) Whetz. It is, therefore, concluded that *B. fuckeliana* is established as the perfect state of *B. cinerea*.

TAXONOMY

The genus *Botryotinia* was erected by Whetzel (**1945**) with *B. convoluta* (Drayt.) Whetz. as the type species. It is known that Professor Whetzel would have liked to designate *Peziza fuckeliana* as the type of *Botryotinia* but refrained from doing so because of the doubt that still existed concerning the connection between it and *Botrytis cinerea*. The choice of *Botryotinia convoluta* does not alter the generic concept in any way since this species and *B. fuckeliana* are very closely related and certainly congeneric.

It should also be pointed out that, although we have been able to show that our isolates from apples, potatoes, and celery will cross with isolates from *Vitis* from Switzerland, and that apothecia resulting from such crosses are morphologically similar to the type of *Peziza fuckeliana*, it does not necessarily follow that all other isolates of *Botrytis* species of the *cinerea* type belong to *Botryotinia fuckeliana*. The fact that isolates from these varied hosts and widely separated geographic localities are interfertile, suggests that the bulk of the isolates of *Botrytis cinerea* will prove to belong to this species. However, it is quite probable that further work will make it possible to recognize specific entities in this great *B. cinerea* complex.

This makes it desirable to fix more accurately the concept of *Botrytis cinerea*. This species was described by Persoon (**1801, 1822**) and the name was accepted by Fries in the Systema Mycologicum (**1832**). An amendment to the International Rules of Nomenclature passed at the VII International Botanical Congress stated that in some cases it may be desirable to accept the type of a pre-starting-point author. This would seem to be a case in which it is desirable to do so.

After the 1950 Botanical Congress, the senior author had the opportunity of examining material in the Persoon Herbarium at Leiden. There were three specimens labelled *Botrytis cinerea,* No. *910.262.835,* No. *910.262.845,* and No. *910.262.847.* No. *835* consisted of a couple of pieces of a grass stem and leaves. There were some blackened areas on the leaves but nothing to suggest sclerotia and no *Botrytis* conidiophores or conidia were found. This specimen would appear to be of no value.

No. *845* consisted of fairly abundant material of some herbaceous stem split and glued to the mounting paper. Conidiophores resembling those of *B. cinerea,* as we understand it, are fairly abundant and sclerotia are present which, although small (about 1–2 mm. in length), are typical *Botryotinia* sclerotia firmly attached to the substrate. One mount was made from this material and typical conidiophores were present, and, although spores were not abundant, some were found measuring 8.5–13 × 6–9 μ.

No. *847* consisted of a small piece of unrecognizable plant material about 3 × 1 cm., bearing *Botrytis* conidiophores but no sclerotia. One mount was also made from this specimen and there were abundant spores 10–15 × 7.5–10 μ.

In view of the fact that in No. *845* the material was more abundant and bore both conidia and sclerotia, this specimen should be taken as the type.

It is, therefore, proposed that No. *910.262.845* in the Persoon Herbarium, Leiden, be designated as the lectotype of *Botrytis cinerea* Pers.

A complete description of *Botryotinia fuckeliana* based on our material is included.

BOTRYOTINIA FUCKELIANA (De Bary) Whetz. Mycologia **37**: 679. 1945.

Peziza fuckeliana De Bary nom. nud. Morph. u. Physiol. der Pilze, Flechten, und Myxomyceten. p. 30. 1866.

Peziza fuckeliana De Bary in Virch. u. Hotzendorf's Samml. gemeinverst. wiss. Vortr. Ser. IV, Heft **87** & **88**: 25. 1869.

Sclerotinia fuckeliana Fuckel, Symb. Mycol. p. 330. 1870.

st. conid.

Botrytis cinerea Pers. Syn. Meth. Fung. p. 690. 1801.
Botrytis cinerea Fries, Syst. Myc. **3**: 396. 1832.
Botrytis fuckeliana Buchw. Den Kgl. Vet.-Landb. Aarsskr. **1949**: 133. 1949.

Sclerotia hard, black, plano-convex and firmly attached to the substrate, very variable in size and shape, rounded to elongated and more or less curved, sometimes convoluted and very irregular, 1–15 mm. in length, sometimes forming confluent masses; in section composed of closely interwoven, hyaline, thick-walled hyphae with a thin, pseudoparenchymatous rind composed of dark brown to black, almost isodiametric cells about 5–10 μ in diameter.

Apothecia arising from the sclerotia singly or in clusters, long stipitate, at first deeply concave or cup-shaped to funnel-shaped, expanding and becoming plane and finally convex, often umbilicate, 1.5–7.0 mm. in diam. and 3–10–(15) mm. in height, the stipe rather stout, 0.5–1.5 mm. in diameter, tapering downwards slightly, the disc avellaneous to wood-brown or verona brown to bister with a delicate, paler margin, the stipe concolorous becoming darker below to blackish at the base, nearly glabrous or minutely pubescent; tissue prosenchymatous, in the stipe composed of hyaline, more or less vertically parallel to slightly interwoven, septate hyphae about 5–10 μ in diameter, at the outside slightly narrower and more compactly arranged and faintly brownish, but scarcely differentiated into a definite excipulum, in the hypothecium the hyphae becoming more intricately interwoven and at the outside a pseudoparenchymatous excipulum is differentiated, composed of large, hyaline, elongated to nearly isodiametric or polygonal cells mostly 10–20 μ in diameter but sometimes up to 60 μ in length, subhymenium differentiated as a narrow, brownish zone of closely interwoven hyphae about 2.5–5.0 μ in diameter; asci cylindric with a long, tapering stalk, eight-spored, (105)–120–140–(160) × 6.5–9.5 μ; ascospores hyaline, one-celled, ellipsoid to ovoid, uniseriate, 9–12 × 4.5–6.0 μ; paraphyses hyaline, filiform, septate, unbranched or very occasionally so, 2.0–2.5 μ in diameter with the tips sometimes slightly swollen.

Conidiophores arising from the mycelium or from the sclerotia, erect, brown, septate, simple or usually branched toward the tip, the tip rounded or slightly swollen, 5–22 μ in diameter, 1–3 mm. in height; conidia grayish-brown in mass, nearly hyaline under the microscope, ellipsoid to ovoid, one-celled, borne on small stalks arising from the tips of the conidiophores and forming botryose clusters, 8–14–(18) × (5)–6–9 μ.

Spermatia hyaline, globose, one-celled, 2.0–2.5 μ in diameter, produced endogenously from hyaline, flask-shaped phialides which may arise singly from the mycelium or more commonly are aggregated into clusters forming spermadochia.

HOST: *Vitis, Pyrus, Solanum, Apium* and probably many other plants.

TYPE: Slide in British Museum, *Peziza fuckeliana* 22/10/64. (Gregory (**1949**)): st. conid. Persoon Herb. *910.262.845.*

SPECIMENS EXAMINED: Apothecia developed in culture, DAOM *19489, 19490, 19491, 19492, 19493, 19494, 19495, 28495, 28496.*

SUMMARY

The connection between *Botryotinia fuckeliana* and *Botrytis cinerea* has been established by cultural and morphological studies and a lectotype of *B. cinerea* has been designated.

ACKNOWLEDGMENTS

The authors are indebted to Dr. P. H. Gregory for his kindness in comparing our material with the type of *Peziza fuckeliana,* to Dr. L. Ettlinger and Dr. S. Blumer for providing cultures of *Botrytis cinerea* from *Vitis,* to Dr. H. J. Lam and Dr. R. A. Maas Geesteranus for permission to examine material in the Persoon Herbarium, and to Dr. F. L. Drayton for his continued interest in the problem and helpful suggestions.

DEPARTMENT OF AGRICULTURE,
OTTAWA, CANADA

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A NEW GENUS OF THE MUCORALES

S. B. Saksena

(with 50 figures)

The writer has been engaged for the past few years in the study of the fungal flora of some forest soils in the neighborhood of Sagar, in Madhya Pradesh State, India. The fungus described below was observed in cultures of soil from the Patharia forest first in October 1950 and again in April 1952. Only the sporangial stages have been observed.

MATERIAL AND METHODS

The soil from Patharia collected in October was somewhat moist. Samples were taken from a depth of about 4" with the help of a sterile spatula, packed in sterile containers and brought to the laboratory.

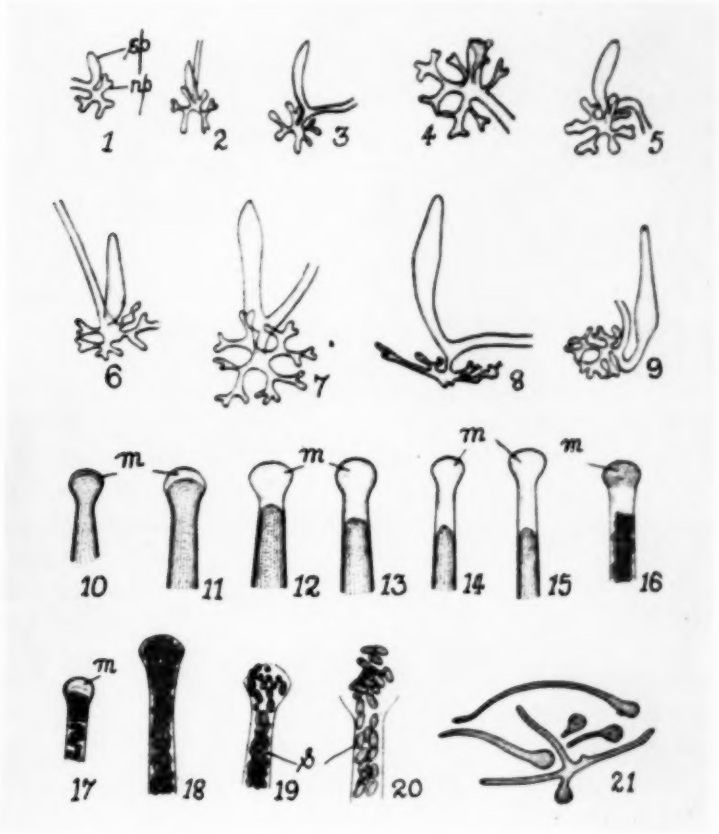
Preliminary isolations were made in Petri dishes with plain agar, Waksman's agar (Waksman **1927**) and potato-dextrose-agar using the dilution technique (Waksman **1927**). The fungus first appeared in Petri dishes with potato-dextrose-agar. Further isolation and purification for purposes of detailed study were made in plain agar and potato-dextrose-agar. Single spore isolations were made by transferring single germinating spores with the help of a stereoscopic microscope.

STRUCTURE OF THE MYCELIUM

The mycelium is fast-growing and covers the entire Petri dish within a week from inoculation. It consists of a large portion of much branched submerged hyphae and profuse flocculent aerial hyphae. The hyphae are 3.2–6.4 μ thick, generally aseptate, but with a few septa in older hyphae. Mycelium treated with chlorzinc-iodide does not give a reaction for cellulose.

DEVELOPMENT OF SPORANGIUM

The fungus was for a long time sterile and no reproductive structures were seen to develop in it. But when pieces of potato-dextrose-agar with the growing mycelium were floated in sterile distilled water, sporangia were formed abundantly. During the



FIGS. 1–9. Earlier stages in the development of the sporangium. (Sp = Sporangial primordia, rp = rhizoidal primordia.) × 860. FIGS. 10–15. Stages showing the formation of apical portion of neck with mucilage plug (m). × 1370. FIGS. 16–20. Stages showing the gradual dissolution of the apical mucilage (m) and the liberation of spores (s). × 860. FIG. 21. Germinating spores. × 860.

past year and more, sporangia have been observed in solid agar medium only a few times and that sparingly. Hence the formation and development of the sporangium have been followed by floating pieces of agar on which the fungus was growing in sterile water.

The sporangia are generally formed at the extremities of short unbranched hyphae. These fertile hyphae are usually found floating on the surface.

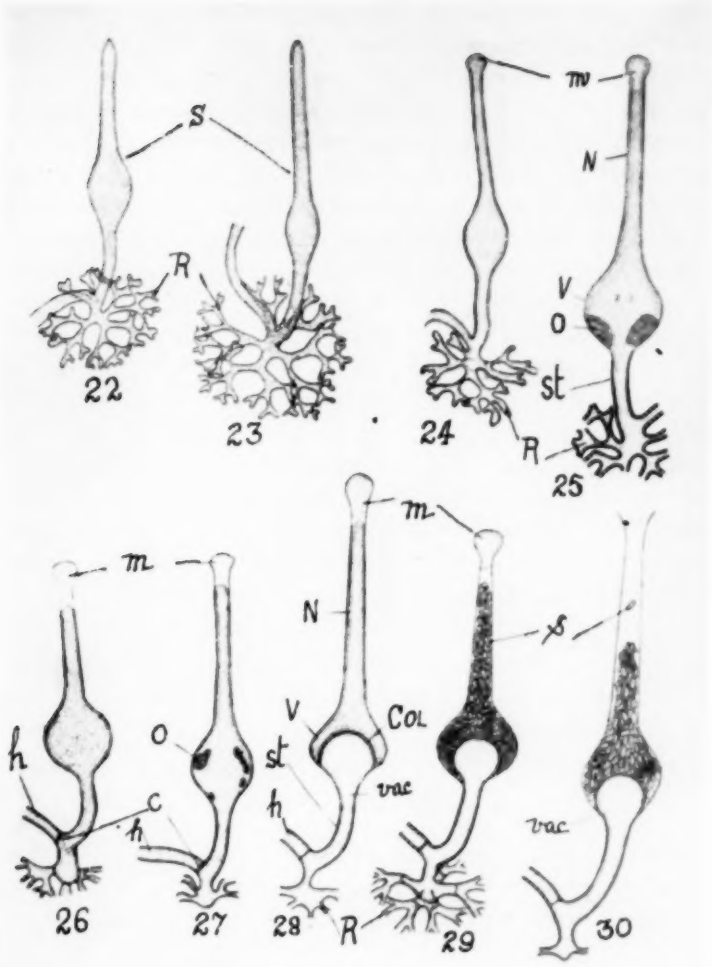
From the end of the sporangium-forming hypha is produced a strongly dichotomously branched rhizoidal complex. A little above the place of origin of the rhizoids a small protuberance is put forth in the opposite direction which ultimately develops into a sporangium (FIGS. 1, 39, Si).

The complex of rhizoidal branches shows some radial symmetry in one plane. These branches are at first aseptate. Later, however, the main branches of the rhizoidal system are each cut off by a septum from the common place of origin (FIGS. 29, 40).

The sporangial primordium (FIGS. 1, 2, 3) at first becomes long and is broader than a normal hypha. The apex at this stage is tapering (FIGS. 39, 40). Later, a portion of this long developing primordium a little above the place of origin slowly swells out into a spherical venter-like structure. The small portion between this venter and the place of origin elongates a little more and becomes the stalk of the sporangium.

Simultaneously with the development of the venter, the upper portion elongates further to form a long neck, the end of which becomes a little bulged out (FIGS. 8, 9, 22–24, 41). The apex becomes rounded on the exterior. Thus the sporangiophore, at this stage, consists of the rhizoidal system below, a stalk, a venter, and a neck with a swollen, somewhat rounded apex. The venter and the neck together give it the appearance of a round-bottomed flask (FIGS. 25–30, 43).

During the increase in size of the sporangium, the contents become very dense. With the increase in the size of the developing sporangium the contents also increase in quantity and keep pace with it, completely filling up the cavity. Later, as the neck elongates further and the tapering apex swells up, the protoplasmic



FIGS. 22–27. Further development of the sporangium (S) with clear differentiation of the rhizoidal complex (R), stalk (St) of the sporangium, venter (V) and the neck (N). ×860. *25, 27.* Show oil globules (O). *26, 27.* Stages showing the cutting off of the sporangium from the parent hypha (h) by a septum (c). FIG. 28. A fully formed sporangium with a columella (Col.), rhizoidal complex (R), venter (V) and the neck (N). FIGS. 29–30. Formation and liberation of spores from the ripe sporangium. ×1370. (S = spores; vac = vacuoles.)

contents do not increase any more and there is a gradual secretion of a dense mucilaginous substance at the apex, which gives pectic reactions but gives no cellulose reaction with chlor-zinc-iodide (FIGS. 10–15, 42, 44). At maturity the apex of the sporangium is occupied by a dense refractive substance forming as it were a sort of plug (FIGS. 44, 49). At this stage both the sporangium and the rhizoidal complex appear as if occupying the upper and the lower ends respectively of a short vertical hypha, and the parent hypha itself appears as if it were a side branch of this vertical hypha (FIGS. 26–29, 50). This parent hypha is soon cut off by a septum. The protoplasmic contents in the sporangium at this stage are very dense and show large oil globules. Soon a dome-shaped columella is differentiated in the venter. The contents of the columella and the stalk are of a thin consistency and soon large vacuoles appear in them (FIGS. 28, 30).

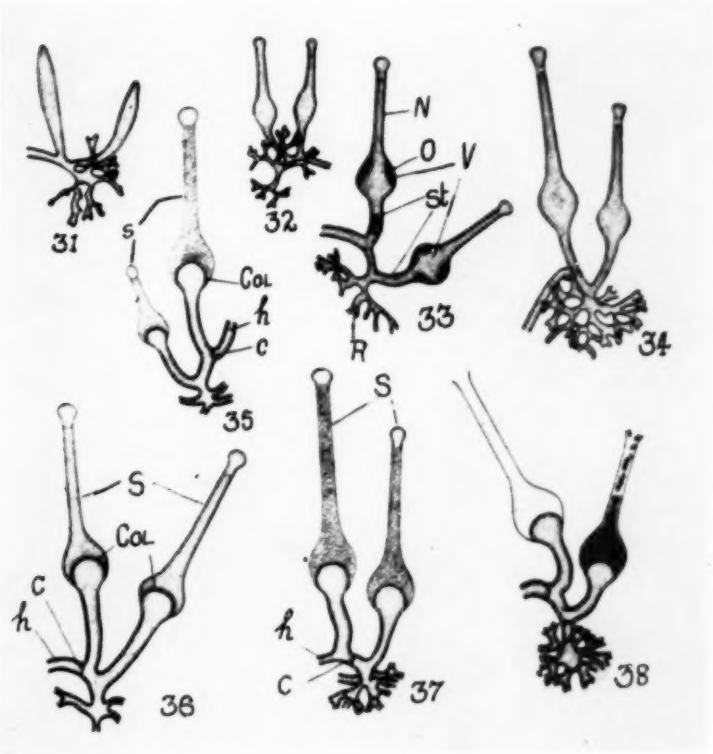
The wall of the sporangium is generally thin, smooth and hyaline and no calcium deposit has been observed. However, the walls of the rhizoidal complex and of the stalk become thickened and somewhat brownish in color.

Usually a single sporangium is developed over a rhizoidal complex at the ends of the sporangia-forming hyphae, but sometimes two sporangia are developed from two primordia formed above a single rhizoidal complex (FIGS. 31–38, 47). These two primordia may be formed either simultaneously or successively. In the former case, the septum cuts off both sporangia from the rest of the original hypha, which therefore lie between the septum and the rhizoidal complex (FIGS. 36, 38). In the latter case the septum is formed between the two sporangia (FIG. 37) and it seems that the sporangium which is proximal to the rhizoids is the older of the two.

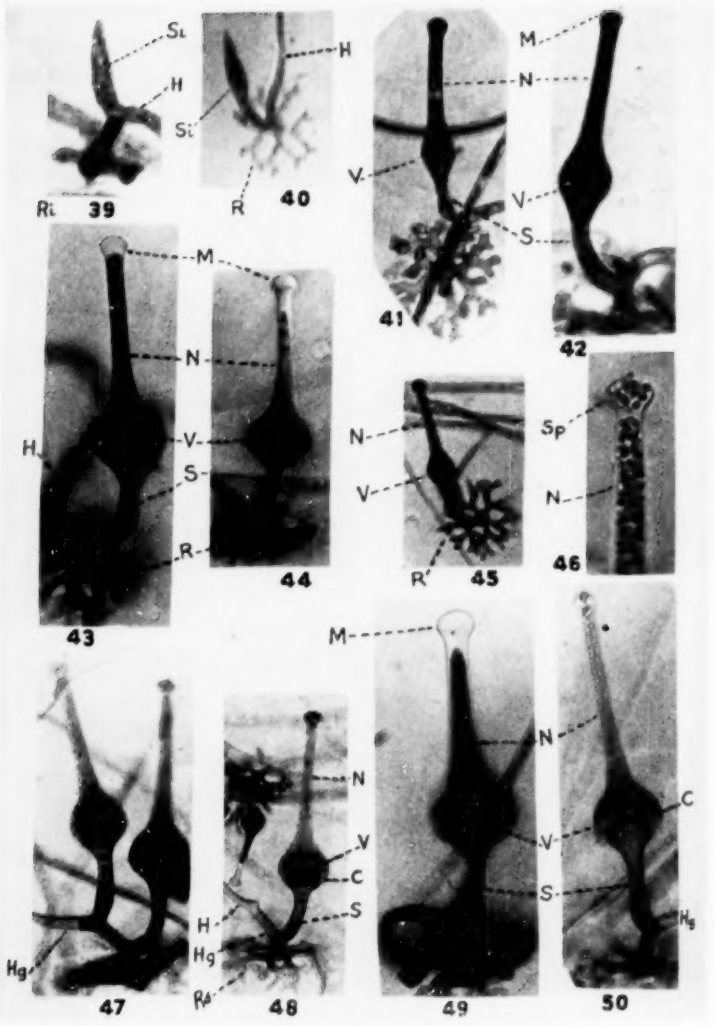
The protoplasm of the venter above the columella is now divided into a number of tiny protoplasts by the formation of cleavage furrows (FIG. 44). The tiny protoplasts become a little bigger, secrete a wall and are thus transformed into spores. These spores come to occupy the entire sporangial cavity (FIGS. 16–18, 48–50). The mucilaginous plug gradually becomes dissolved away and finally the thin wall at the convex apex is also gelatinised. The

spores are then discharged through the dissolved apex of the sporangium (FIGS. 19, 20, 30, 38, 46). The expulsion is brought about by a passive movement of the spores through the neck in a series.

These liberated spores seen to accumulate outside the neck and do not show any movement. Often, some spores were seen to



FIGS. 31–38. Stages in the formation and development of double sporangia. × 860. 31–34. Stages leading to the formation of the sporangium and differentiation of the venter (V) and the neck (N). (O = oil globules; R = rhizoidal complex.) 35–36. Two fully formed sporangia with columella (col) and a septum (c) cutting off both the sporangia (S) from the parental hypha (h). 37. Two fully formed sporangia (s) with a septum (c) formed in between the two sporangia. 38. Showing two sporangia, in one of which the spores are being liberated; the other is empty.



FIGS. 39–43, 45. Formation of the sporangium. 39. Young sporangium initial. 40. Further growth of the sporangium initial. 41. Differentiation of the venter and the neck. 42, 43, 45, 49. Further development of the venter, differentiation of the apex and secretion of the mucilage-like substance. FIGS. 44, 48, 50. Fully-formed sporangia with columella and spores. 44. Formation of distinct protoplasts by the sporangial contents. 48, 50.

remain inside the venter of the sporangium itself and to germinate *in situ*. The spores at the time of liberation are oblong in shape. The liberated spores were transferred to Czapek's liquid medium for germination. During germination the spores swelled and became spherical, sub-spherical or ovoid. Usually one germ tube was produced (FIG. 21).

Sexual reproduction in this fungus has not been observed so far in any of the cultures.

This fungus with its non-septate hyphae and aerial sporangia containing aplanospores clearly belongs to the Mucorales. The presence of a distinct columella and the fact that the sporangia are all of the same shape (i.e., there are no sporangiola or "conidia") and lack heavily cutinized walls indicate a relationship to the family Mucoraceae. On the other hand, the dichotomously branched hyphae (which probably act as props) at the base of the sporangiophores, suggest a kinship to the genus *Syncephalis*, some species of which have remarkably similar structures. The present genus, because of the flask-shaped sporangia, their dehiscence by the dissolution of an apical plug and the method of spore discharge, does not seem to fit into any of the present families of the Mucorales. Its position within the order can best be determined after the sexual reproductive stages have been found.

It is proposed to erect a new genus. The genus is named, *Saksenaea*, after Dr. Ram Kumar Saksena of the Allahabad University, under whose guidance and advice the writer is working. The type species, which at present is the only one known in this new genus, may be called *Saksenaea vasiformis* sp. nov.

Later stages in the formation and liberation of spores; apical plug has been dissolved and only the wall is intact. 46. Apex of a mature sporangium showing the apical opening through which the spores are liberated. 47. Two sporangia formed from the same parent hypha. (*R* = Rhizoid complex; *Rs* = Rhizoidal septum; *Ri* = Rhizoidal initials; *S* = Stalk of the Sporangium; *Si* = Sporangium initial; *V* = Venter; *N* = Neck; *H* = parental hypha; *Hs* = Septa cutting off the parental hypha and the Sporangium; *M* = mucilage-like substance; *C* = Columella; *Sp* = Spores.) (Magnifications: 39, × 650; 40, × 250; 41, × 375; 42 & 49, × 460; 43, × 550; 44, 47, 50, × 500; 45, × 300; 46, × 900; 50, × 350.)

Saksenaea gen. nov.

Sporangia urceolata, ventre sphaerico distincto atque collo longo, insidentia singula vel bina apicibus hypharum aerearum, sporangiophoris singulis supra massam rhizoidalem saepe bifurcatam positis. Columella prominens atque cupulae similis. Sporangia dehiscentia per dissolutionem obstructionis vel operculi apicalis mucilaginosi; sporae dispersae per hoc collum.

Sporangia flask-shaped with a distinct spherical venter and a long neck; formed singly or in pairs at the ends of aerial hyphae, each sporangiophore above a profusely dichotomously branched rhizoidal complex. Columella prominent and dome-shaped. Sporangia dehiscing by the dissolution of an apical mucilaginous plug, spores discharged through this neck.

Saksenaea vasiformis sp. nov.

Mycelium bene evolutum, cito crescens hyphis ramosissimis productis duplicis speciei, quarum una submersa atque profuse ramosa, altera vero flocculenta atque aerea 3.2–6.4 μ lata. Sporangia ut plurimum singula, nonnumquam bina, erecta, insidentia rami hyphalis apici, qui infra structura rhizoidea dichotome ramosa est ornatus, rhizoideis 3.2–4.8 μ, latis, pedicellata, pediculis 6.4–9.6 × 24–64 μ, urceolata ventre sphaerico, 16–43.2 × 22.4–51.2 μ, columella distincta cupulae simili praedita; venter collo longo superposito 6.4–11.2 × 54.4–200 μ ornatus; colli apex paululo latior 8.0–14.4 μ diam., obturamento mucilaginoso occlusus. Sporangiosporae parietibus praeditae, oblongae, 1.4–2.1 × 2.8–4.2 μ, per collum obturamento apicali dissoluto emissae; in germiatione ut plurimum unus germinationis tubulus efformatus.

Typus lectus in terra, in loco Patharia Forest, in Sagar, in regione Madhya Pradesh, in India.

Mycelium well developed, fast-growing with much-branched hyphae of two kinds, one submerged and profusely branched, the other flocculent and aerial, 3.2 to 6.4 μ broad. Sporangia generally single, sometimes in twos, erect, developed at the end of a hyphal branch with a dichotomously branched rhizoidal structure below, rhizoids 3.2 to 4.8 μ broad, stalked, stalk 6.4–9.6 × 24–64 μ, flask-shaped with a spherical venter, 16–43.2 × 22.4–51.2 μ, with a distinct dome-shaped columella; venter surmounted by a long neck, 6.4–11.2 × 54.4–200 μ, apex of the neck slightly broader, 8.0–14.4 μ in diameter, closed with a mucilaginous plug. Sporangiospores walled, oblong, 1.4–2.1 × 2.8–4.2 μ, discharged through the neck by the dissolution of the apical plug; during germination usually one germ tube formed.

In soil, Patharia forest in Sagar, Madhya Pradesh State, India.

The peculiar and characteristic shape of the sporangium recalls to mind the sporangia of *Nephrochytrium*, *Catenomyces* and

Rhizidiomyces (see Karling **1944**, Hanson **1945** and Bessey **1950**, Figs. 18 and 22D). In these fungi the sporangia have distinct necks through which zoospores are liberated. It will be interesting to note here that in a species of *Catenomyces* the apex of the neck shows an accumulation of a thick viscid substance (mucilage ?) prior to dehiscence as in the case of the present fungus. This resemblance does not go very far as these three fungi are zoosporic forms and do not have nonmotile endospores as in the present fungus. In addition to this, the sporangium in the present fungus has a distinct and well-formed columella.

On the other hand this mode of sporangial dehiscence with a definite neck does not occur in any of the known Mucorales. This strange resemblance to the chytridiaceous members reminds one of the suggestion of von Jaczewski (1929–30) that the Zygomycetes may have been evolved from the chytridiaceous fungi (see also Bessey **1950**, p. 635 and Fitzpatrick, **1930**, p. 47). In the case of the present mucoraceous fungus this would have involved two steps: (1) the formation of a columella and (2) the formation of nonmotile sporangiospores instead of zoospores.

SUMMARY

A new mucoraceous fungus isolated from a forest soil in India is described in detail.

The fungus is characterized by a stalked flask-shaped columellate sporangium. A large number of sporangiospores are formed and are liberated through the neck by the dissolution of an apical mucilaginous plug.

This fungus is quite unique and does not agree with any of the known genera and hence it is placed in a new genus, *Saksenaca*, the fungus itself being named *Saksenaca vasiformis*.

The affinities of the genus are briefly discussed.

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DEPARTMENT OF BOTANY,
UNIVERSITY OF SAUGAR,
SAGAR, M. P., INDIA

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STUDIES OF SUB-TROPICAL RUSTS. I. PROSPODIUM TRANSFORMANS [1]

FLOYD S. SHUTTLEWORTH [2]

(WITH 9 FIGURES)

Although the Uredinales have been studied cytologically since the publication of Schmitz's (**23**) paper describing the binucleate condition found in the mycelium and urediospores of *Colcosporium campanulae* (Pers.) Lév., there is much yet to be learned concerning the morphology and nuclear behavior exhibited by this important group of obligate parasites. Especially have the tropical and sub-tropical species been neglected. In the opinion of Jackson (**16**) and most other modern students of the Uredinales, the short-cycle forms are considered to be the most recently derived species of rusts and thus are the expression of the present day evolutionary trend. Accordingly, there is greater variation among the microcyclic species than among the macrocyclic ones.

In order to determine whether the sub-tropical short-cycle forms exhibit the same degree of variation as do the temperate forms which have been investigated, a study was begun of several sub-tropical microcyclic species, including *Prospodium transformans* (Ell. & Ev.) Cumm., a parasite of *Tecoma stans* (L.) HBK. This rust occurs in South Florida and throughout the West Indies.

[1] This paper is a portion of a thesis submitted to the Faculty of the Graduate School in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Botany, Indiana University, November, 1950.

[2] The author wishes to express his gratitude to Dr. T. R. Alexander, Mr. M. J. Soule, Jr., and other members of the staff, Department of Botany, University of Miami; to Mr. Donald Lawrance, Nassau, Bahamas; to Dr. G. B. Cummins, Arthur Herbarium, Purdue University; and to Dr. H. J. Brodie, Department of Botany, Indiana University.

MATERIALS AND METHODS

Material of *Tecoma stans,* infected with *Prospodium transformans,* was collected at Coral Gables, Florida, and Nassau, New Providence, Bahamas. Collections were made in Florida throughout the year and in the Bahamas during July and December. Leaf, stem, and fruit specimens were obtained.

A number of solutions were used as fixatives, including acetic-acid-alcohol, Bouin's, Randolph's modification of Navashin's fluid (CRAF) (**18**), FAA (**22**), and Flemming's fluid, weaker solution (**18**). The last two fixatives proved to be the most satisfactory for this study. After dehydration with tertiary butyl alcohol (**18**), the material was imbedded in paraffin and was sectioned on the rotary microtome at 5, 7, 10, and 15 μ. Fresh material was also cut on the freezing microtome at 10 and 15 μ, using a razor blade holder with a single edge blade instead of the special knife ordinarily used with the freezing microtome. For the large galls formed by *P. transformans,* the freezing microtome method was more satisfactory than the paraffin method.

Of the various staining techniques employed, Tuan's (**26**) iron-alum hematoxylin-picric acid method, Harris' hematoxylin (**18**), Flemming's triple stain (**18**), and the Feulgen reaction (**10**) gave the most satisfactory results.

For the study of germination, the spores were placed in a drop of water on a slide which was inverted over two glass rods in a petri dish containing filter paper soaked with distilled water. Better permanent mounts were obtained by first placing a thin film of 1% water agar on the slide (**12**) and adding a drop of spore suspension after the agar had dried. In both methods, after germination had taken place, the slides were air-dried, fixed with CRAF, FAA, or osmic acid fumes and then stained with aceto-carmin for immediate study or with one of the two hematoxylin methods or with the Feulgen reaction for permanent mounts.

For the inoculation studies, small plants of *Tecoma stans,* potted in good soil, were sprayed with a fine mist of distilled water, placed in an airtight glass container which had also been sprayed with distilled water, and inoculated with teliospores germinated in a petri dish of distilled water. Control plants were treated in the same manner except that the spore inoculation was omitted.

RESULTS AND DISCUSSIONS

Prospodium transformans parasitizes two species of *Tecoma* (*Stenolobium*) throughout its geographic range. This rust is distributed from southern Florida and the Bahamas to Mexico, Cuba, and Guatemala (**9**). A microcyclic species which forms only pycnia and telia, it is considered to be correlated with the macrocyclic *P. appendiculatum* (Wint.) Arth. (**9**). In Florida, *P. transformans* and *P. appendiculatum* usually occur on the same host plant, the telia of the former and the uredia of the latter species often on the same leaf. However, during the three years of this investigation, the author did not observe the pycnial-aecial gall-forming stage of the macrocyclic species in Florida, although the uredial stage was abundant and the telial stage not too uncommon.

Pycnia of *P. transformans* are amphigenous, caulicolous, or fructiculous, yellow-brown in color, subcuticular in position, and conical in shape. They are often overlooked as they are soon replaced by the telia. Pycniospores are spherical in shape and uninucleate.

The telia may cause slight to severe hypertrophy on leaves, or even extensive galls (FIG. 1) on petioles, stems, and fruits. The galls reach a diameter of over 3 cm. and are more or less spherical or sometimes longer than wide. Internally the galls are composed of greatly enlarged parenchymatous cells, which have increased not only in size but in number as well. The vascular tissue is small and scattered throughout the gall. Galls can occur on *Tecoma* anywhere from the base of the stem to just below the terminal bud. Because these galls are composed of such thin-walled cells, the stems which are so attacked are prone to break off during hurricanes and severe wind storms. This may be one of the principal reasons why *Tecoma stans* seldom attains tree size in Florida, remaining usually a shrubby plant with many main stems arising from the base. The interior of the gall is packed with intercellular hyphae which have prominent haustoria, often coiled as described by Evans (**13**) and Rice (**21**) for other rust species.

Teliospores are borne on the surface of the hypertrophied areas or on the entire surface of the galls. Sometimes the teliospores

are borne internally within the gall itself. Internal sori have been reported for a number of species (**1**, **29**), and Colley (**8**) has termed them "teratologic phenomena of no special morphologic significance."

The teliospores are leptosporic; that is, they germinate immediately upon maturation if the environmental conditions are satisfactory. A high humidity is necessary for germination. However, when all other conditions are satisfactory, temperature is the limiting factor. Suspensions of spores kept at 10° C did not germinate, and only one to two per cent germinated at summer room temperature (27°–31° C). About ninety per cent germination was obtained at 18°–21° C. Exposure to this temperature range for only one or two hours and then removal to room temperature did not decrease the amount of germination. Cochrane (**7**) found that brief exposure to optimum temperatures materially increased the germination of urediospores of a species of rust held at either high or low temperatures. Suspensions of teliospores of *P. transformans* kept for 24 hours at either 10° C or at room temperature germinated abundantly when transferred to an airconditioned room with a temperature range of 18°–21° C. This temperature range appears to be near the optimum for this species. This temperature is slightly lower than that found for teliospores of *Endophyllum heliotropii* Thirum. and Navasimhan (**25**) and corresponds more nearly to that found as optimum for the urediospores of a number of species in northern areas (**7**, **14**, **27**).

The teliospores of *P. transformans* are normally two-celled and break off from the pedicel near the base of the spore. The germ pore is apical in the upper cell and is next to the pedicel in the lower cell. A few three-celled teliospores were observed. In such spores the lower cell was normal, the upper two cells separated by a vertical septum. The upper two cells were each near normal size, with germ pores lateral, although actually apical in relation to the vertical cross wall. The multiplication of cross walls is, according to Arthur *et al.* (**1**), of common occurrence in the Pucciniaceae, but such variation in the structure of the spore, due to number and position of septa, may be considered as abnormal development and of no special significance.

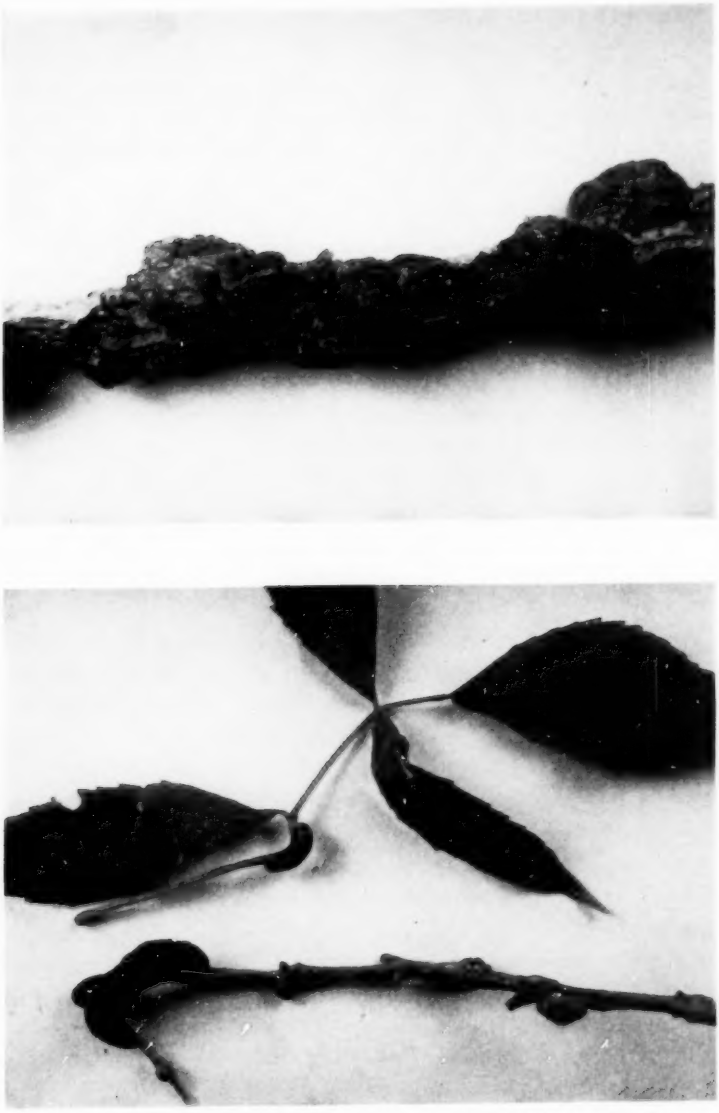
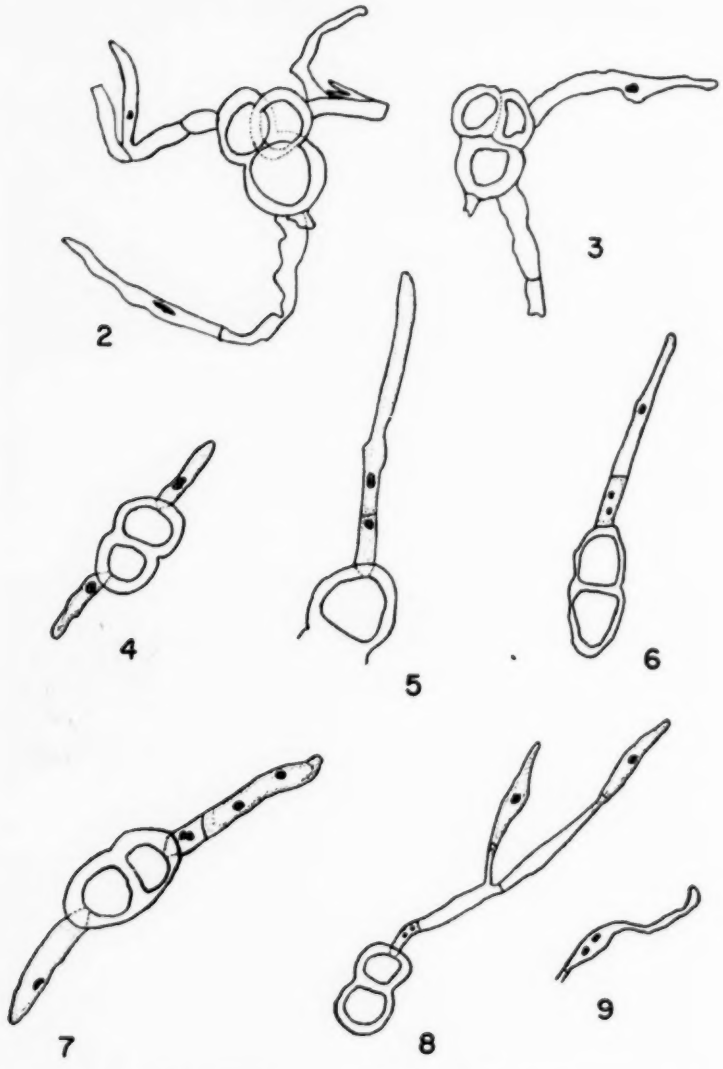


FIG. 1. Galls formed on *Tecoma stans* as result of infection by *Prospodium transformans*. Upper photograph, main stem in which galls have nearly girdled the stem. Lower photograph, galls on young twig and on leaf petiole. These galls are covered with mature teliospores.



FIGS. 2–9. Germinating teliospores of *Prospodium transformans*. 2. A three-celled teliospore showing all three cells germinating. Germination abnormal. 3. A three-celled teliospore with two cells germinating. Portion of one tube broken off. 4. Normal two-celled teliospore, both cells germinating, each showing the fusion nucleus which has migrated out into

Although there have been reports of teliospores having supernumerary cells, there have not been, apparently, any reports concerning their germination. FIG. 2 shows one such spore in which all three cells have germinated, the upper left teliospore cell having a definite two-celled basidium, with one somewhat abnormal basidiospore and the other cell of the basidium broken off. FIG. 3 illustrates one three-celled spore in which two cells have produced germ tubes.. The end of one tube was broken off in the preparation of the slide.

NUCLEAR BEHAVIOR

The young teliospores of *Prospodium transformans* are binucleate and arise from binucleate cells at the base of the sorus. The mature teliospore is uninucleate indicating that a fusion has taken place. This is the only true diploid uninucleate cell in the life cycle of this rust. At what place and in what manner the binucleate condition arises has not been determined. The pycnia and the pycniospores are uninucleate, and most intracellular hyphae also appear to be uninucleate. No evidence of fusion cells or nuclear migrations, as reported (**2**, **6**, **28**) for other species of rust, was found. Olive (**19**) reported fusion cells for *Prospodium* (formerly *Puccinia*) *transformans*, but, according to Jackson (**16**), the species with which Olive was working was *P. elegans* (Schroet.) Cumm. and not *P. transformans*. The binuceate condition in *Puccinia malvaccarum* Bert. was reported by Ashworth (**2**) and by Brown (**4**) as arising from a nuclear division without cross wall formation, and thus the two nuclei are sister nuclei and not a true dicaryon. This latter species is, according to Brown (**4**), homothallic. Although attempts were made to obtain single basidiospore infections with *Prospodium transformans*, none were obtained, and, therefore, it is unknown whether this species is

the tube. 5. Germ tube with one nucleus in stalk cell and one nucleus in basidial initial. 6. Germ tube in which nucleus of stalk cell has divided but nucleus in basidial initial has not. 7. Teliospore with a 4-nucleate germ tube and a 1-nucleate germ tube. 8. Mature two-celled basidium. Two nuclei in stalk cell starting to degenerate. Basidiospores uninucleate. 9. Binucleate basidiospore which has started to germinate while attached. Nuclear division precocious. Cytoplasmic contents not shown.

homothallic or heterothallic. The presence of well-developed pycnia suggests that this species is probably heterothallic.

Upon germination, the teliospore produces a two-cell basidium, supported by a very short basidial stalk cell. Each cell of the basidium proper produces a single basidiospore, uninucleate at first, but often becoming binucleate by mitotic division as the spore begins to germinate *in situ*. Two-celled basidia are known for several species of rust, but in most cases the nuclear behavior in such species is unknown. The species in which the nuclear behavior has been studied are discussed by Jackson (**17**).

The first meiotic division of the fusion nucleus may take place within the teliospore but more commonly occurs within the germ tube, usually near its point of emergence from the spore. FIG. 4 illustrates the fusion nucleus which has migrated into the germ tube but has not yet divided. FIG. 5 shows the result of the first meiotic division in which the two nuclei are separated by a septum before undergoing the second meiotic division. In some cases the second division appears to occur in both nuclei at approximately the same time: in other cases (FIG. 6) the nucleus in the stalk cell may divide before the one in the basidial initial divides. In either case, two of the resulting four nuclei remain in the stalk cell, where they eventually degenerate, leaving the stalk cell empty of all protoplasmic material.

The second nucleus of the first meiotic division divides again in the basidial initial either before (FIG. 7) or after the sterigmatal branch begins to protrude. This side protuberance ordinarily remains short and acts as a sterigma rather than a true branch. The end portion of it swells into a basidiospore and is densely filled with cytoplasm. The main portion of the germ tube elongates and the upper end constricts and then swells to form the second basidiospore. A cell wall is laid down just above the area where the side protuberance originated. The two nuclei, one in each cell of the basidium, migrate into their respective basidiospores, the one going to the terminal basidiospore often being much drawn out as it passes through the constriction of the sterigma into the spore. Vacuolization at the base of the spores occurs, and a wall is laid down cutting off each spore (FIG. 8).

The basidiospore, while still attached to the basidium, may begin to germinate, sending out a long, narrow, and often curved tube, densely filled with cytoplasm. In such a spore, there may be two nuclei (FIG. 9) which have arisen by precocious division without the formation of a cross wall.

Jackson (**17**) has summarized the six types of variation found in microcyclic rusts. The majority of these are known for Endoforms because in very few Micro-forms has the nuclear detail of germination been studied. The six types of variations as given by Jackson are as follows:

"1. The mycelium is at first uninucleate, becoming binucleate in connection with the development of the sorus plexus. A fusion of the two nuclei occurs in the young teliospore or aeciospore. Meiosis follows, resulting in four nuclei and a four celled basidium and uninucleate basidiospores. . . .

2. The mycelium is uninucleate, becoming binucleate. In the young spore (morphologically the aeciospore) no fusion occurs. The two nuclei pass into the developing basidium and each divides once. Septa are laid down between each of the nuclei and the mature basidium is four celled. Four uninucleate basidiospores develop. . . .

3. The mycelium is binucleate throughout. The nuclei fuse in the young teliospore. In the developing basidium a septum is laid down following the first meiotic division. No septum is formed after the second division. The mature basidium is two celled, each basidiospore receiving two nuclei, the product of the second meiotic division. . . .

4. The mycelium is at first uninucleate, becoming binucleate. The young teliospore is binucleate but no fusion occurs. One of the two nuclei degenerates. The remaining nucleus passes into the promycelium and is isolated by a cross wall. It then divides once. A cross wall is laid down between the daughter nuclei. The mature basidium is two celled but a basidiospore is formed only from the upper cell, the nucleus in the lower cell degenerating. . . .

5. The mycelium throughout is uninucleate as well as the young teliospore. In the basidium the single nucleus divides once and a septum is laid down between the resulting nuclei. The mature basidium is two celled, the basidiospores uninucleate. . . .

6. The mycelium is uninucleate throughout, the nucleus of the young teliospore passes into the basidium and divides twice. The mature basidium is four celled and the basidiospores uninucleate. . . ."

To these six variations, Thirumalachar and Narasimhan (**25**) have added a seventh type found in *Endophyllum heliotropii*. In this rust the mycelium is binucleate throughout. The teliospores are binucleate and the nuclei do not fuse, but move out into the basidium where they do not divide. They are separated by a septum, forming a two-celled basidium. The nuclei move into the basidiospores where they divide, forming binucleate basidiospores which give rise to a binucleate mycelium.

To these seven variations must now be added an eighth variation, namely that found in *Prospodium transformans*, described in this paper. The mycelium is uninucleate, becoming binucleate. The young teliospores are binucleate, but fusion occurs in the mature spore. Meiosis results in four nuclei, two of which degenerate within the basidial stalk cell. A two-celled basidium is cut off, and each basidiospore is uninuculeate.

From this summary it is evident that the microcyclic species of rusts exhibit great variation, and further studies, especially of the tropical and sub-tropical forms, will probably show that still other variations occur.

CELLULAR INCLUSIONS

In the rust infected tissue of the leaves of *Tecoma stans*, the abundance and size of the cellular inclusions were so striking that certain cytochemical tests were made to determine their nature. The physical nature of these inclusions and the manner in which they coalesce suggest the coacervates described by Bungenberg de jong (**5**), Dobry (**11**), Humphrey and Dufrenoy (**15**), Reed (**20**), and others, although they did not give the color reactions which the above workers obtained with the molybdenum reagent. The inclusions were stained by all of the basic stains applied, including the basic fuchsin of the Feulgen reaction. Nuclei and haustoria could often be seen in the same cells with the inclusions. A detailed account of this phase of the present investigation is to be published elsewhere.

INOCULATION EXPERIMENTS

Young plants of *Tecoma stans* apparently free of rust infection were obtained from northern Florida. They were inoculated in

the manner described earlier in this paper, and control checks were given the same treatment except that the spore suspension was omitted. Pycnia and telia appeared in 18 to 21 days in most cases, but one plant produced no visible signs of infection before 35 days. Pieces of infected and control leaves were killed, fixed, and paraffin sections made. Galls did not appear on the stems or petioles during the five months of observation, although the leaves were hypertrophied where the pycnia and telia formed. The control plants exhibited no signs of infection at the end of the observation period.

SUMMARY

1. A sub-tropical microcyclic species of rust—*Prospodium transformans* which parasitizes *Tecoma stans*—was investigated. Material was collected in South Florida and in the Bahamas.

2. A variety of fixing solutions and staining methods was used. Both paraffin sections and freezing microtome sections were made.

3. *P. transformans* causes hypertrophied areas on leaves, and large galls on stems, petioles, and fruits. The host tissue in such areas is parenchymatized and the vascular bundles are modified and scattered within the galls.

4. These galls are packed with intercellular hyphae which have large haustoria within the host cells.

5. Internal sori are often formed within the galls.

6. *P. transformans* produces both pycnia and telia. The former are uninucleate, the latter binucleate at the base of the sorus and in the young teliospores.

7. Fusion takes place within the mature teliospore. Germination of the spore results in a two-celled basidium. The fusion nucleus undergoes two meiotic divisions, and two of the four resulting nuclei remain in the basidial stalk cell where they degenerate.

8. The basidiospores are uninucleate when formed. They begin to germinate *in situ* and often become binucleate by a precocious division of the nucleus.

9. The optimum temperature range for the germination of the teliospores of *P. transformans* is apparently 18°–21° C.

10. The type of nuclear cycle displayed by *P. transformans* differs from all of the six variations described by Jackson and also from the seventh type of variation recently reported by Thirumalachar and Narasimhan.

11. Within the leaves of *Tecoma stans* infected with *P. transformans* are formed cellular inclusions, interpreted to be coacervates of undetermined chemical composition.

12. Inoculations usually produce pycnia and telia after 18 to 21 days. Sometimes longer periods are necessary for visible signs to appear.

DEPARTMENT OF BOTANY,
UNIVERSITY OF MIAMI,
CORAL GABLES, FLORIDA

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A UNISEXUAL MALE CULTURE OF CHALARA QUERCINA [1]

H. L. Barnett

(WITH 3 FIGURES)

Most of the workers who have studied a number of isolates of *Chalara quercina* Henry in culture have noted considerable variation in the cultural characteristics of the isolates. Henry (**3**) was the first to note these differences, which are mainly in appearance and growth rate of mycelium and in abundance of conidia. Bretz (**1**) has shown that the fungus is heterothallic and capable of producing perithecia and ascospores in culture. Hepting *et al.* (**5**) have recently reported that single ascospore cultures fall into two groups designated as A and B. Each single spore culture is typically bisexual (hermaphroditic), capable of acting both as male and as female, self-sterile and cross-fertile. The conidia act as spermatia. In this respect *C. quercina* is apparently quite similar to *Hypomyces solani* f. *cucurbitae* as reported by Hansen and Snyder (**2**).

The present paper reports the results of a portion of a broader study that deals with the crossing of isolates obtained from different states in the oak wilt region. More specifically, this paper reports an isolate of *C. quercina* which has the ability to act as male but which apparently has lost the ability to act as a female.

MATERIALS AND METHODS

All cultures used in the present study originated from single spores of isolates from oak wilt trees. All isolates numbered in the 600 series are from West Virginia. Culture *805* is a transfer from a Minnesota isolate No. *R39* obtained through the courtesy of Dr. D. W. French. It was isolated August, 1950. Cultures *808* and

[1] Published with the approval of the director of the West Virginia Agricultural Experiment Station as Scientific Paper No. 453.

810 are from Iowa, obtained through the courtesy of Dr. W. H. Bragonier.

Considerable time and effort were spent in the selection and improvement of a semisynthetic medium which could be duplicated easily and which would be favorable for the production of perithecia. The medium finally selected had the following composition: maltose 5 g.; Difco Casamino Acids (technical grade) 1 g.; KH_2PO_4 1 g.; $MgSO_4 \cdot 7H_2O$ 0.5 g.; Zn and Fe 0.2 mg.; Mn 0.1 mg. (as sulfates); biotin 5 μg.; distilled water 1 liter; agar 20 g. The pH of the medium should be adjusted to approximately 6.0 before autoclaving at 15 pounds pressure for 15 minutes. If no pure biotin is available the partial deficiency of some isolates may be overcome by adding 0.5 g. yeast extract to the medium.

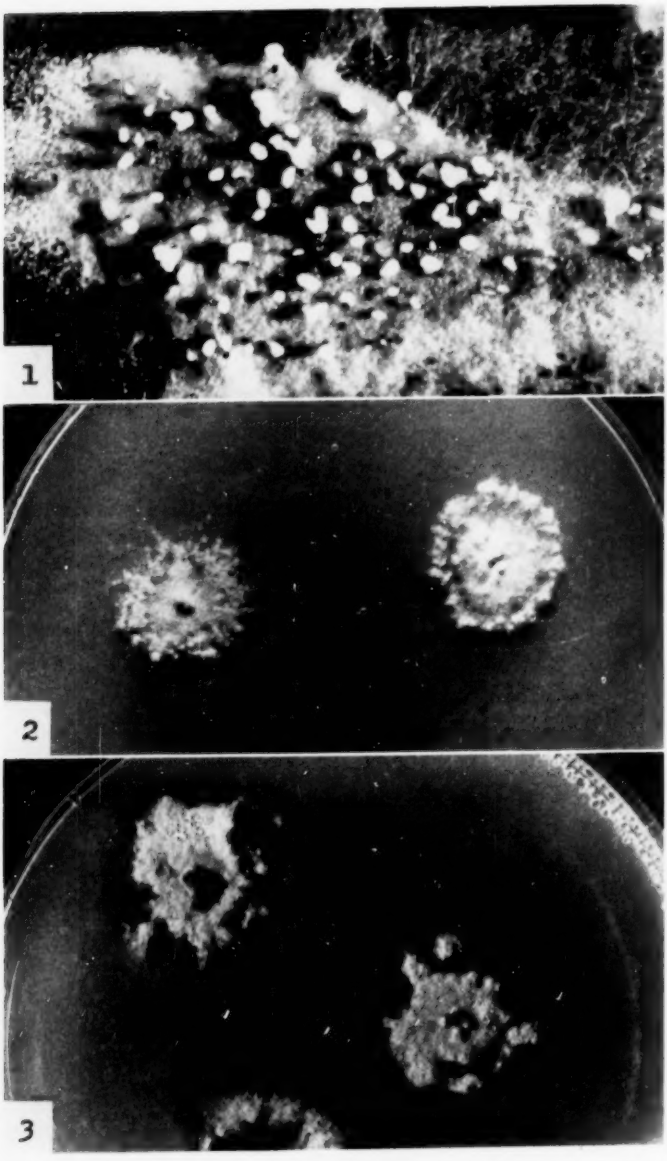
Petri plates of the above medium were inoculated at three points with pieces of mycelium which were allowed to grow for six days at 25° C. At this time the cultures were spermatized by placing loopfuls of conidial suspension on the mycelium or by spraying the mycelium with a spore suspension using an atomizer. Three days later the cultures were examined by means of a stereoscopic wide field microscope. Cultures having no perithecia were examined again two days later. The presence of perithecia producing ascospores indicated a fertile cross. All crosses were repeated once or twice.

EXPERIMENTAL RESULTS

Thirty isolates, each from a different tree in West Virginia, were crossed with four cultures used as standard in order to determine the compatibility group and crossing ability of each isolate. Fourteen were of group B and 16 belonged to group A.[2] All were bisexual, capable of acting both as male and as female, self-sterile and cross-fertile. These results verify the findings of Hepting *et al.* (**4**). The production of perithecia on maltose-Casamino Acids medium is shown in FIG. 1.

Five Minnesota isolates were then used as female cultures in crosses with four West Virginia isolates as male. The results are

[2] For the sake of uniformity A and B are applied to the same compatibility groups first designated A and B by Hepting *et al.* (**4**).



FIGS. 1–3.

given in TABLE I. The presence of fertile perithecia is indicated by P, their absence by O.

TABLE I

RESULTS OF CROSSING MINNESOTA ISOLATES USED AS FEMALES WITH WEST VIRGINIA ISOLATES

Minn. / W. Va.	A *801*	B *802*	B *803*	B *804*	A *805*
B *611*	O	P	P	P	O
B *613*	O	P	P	P	O
A *621*	P	O	O	O	O
A *638*	P	O	O	O	O

Isolate *805* was unique in that it failed to act as female in crosses with 4 West Virginia isolates of either A or B group. Reciprocal crosses showed that all of the Minnesota cultures were capable of acting as males. The crosses between *805* used as male and West Virginia B cultures as female produced perithecia and ascospores. Therefore, *805* belongs to the A group.

The crosses were then expanded to include other cultures from West Virginia, Minnesota and Iowa as male and two single spore isolates of *805* as female. The results are given in TABLE II.

TABLE II

RESULTS OF CROSSING TWO SINGLE-SPORE CULTURES OF *805* AS FEMALE WITH 9 OTHER CULTURES USED AS MALE

	B *801*	A *808*	B *810*	B *611*	A *620*	B *622*	A *624*	B *625*	A *638*
A *805-1*	O	O	O	O	O	O	O	O	O
A *805-2*	O	O	O	O	O	O	O	O	O

These results strongly indicate that isolate *805* is a unisexual male, incapable of acting as a female. Further evidence was obtained from 4 additional single spore isolates of Minnesota *R39* (the source of *805*) made by D. W. French. None of these produced perithecia when spermatized with conidia of test West

FIG. 1. Perithecia and light masses of ascospores produced in culture, × 10.

FIG. 2. Culture *805*, 6 days old. Note the light mycelium with small aerial tufts.

FIG. 3. Culture *633*, 6 days old. Note the dark smooth mycelium.

Virginia isolates. The macroscopic appearance of isolate *805* distinguishes it from most other isolates. The mycelium is light on the above medium and it forms numerous small aerial tufts (FIG. 2). In contrast, the majority of the isolates produce dark mycelium which may be uneven in its growth but does not produce the small tufts. It must be emphasized that the color of the mycelium varies with the medium used. Light and dark mycelia as described in this paper were compared on the maltose-Casamino Acids medium. Apparently they are the same forms which Henry (3) described as "white" and "normal."

If isolate *805* is genetically unable to act as a female, a cross with a bisexual isolate should give F_1 single-ascospore isolate, half of which would be bisexual and half unisexual males. To test this hypothesis, isolate *633* (bisexual, dark mycelium, FIG. 3) was selected as a typical culture to use as female in cross with *805*.

From this cross 23 single ascospore cultures were obtained. It was soon apparent that some of the cultures were like *633* in appearance while others were like *805*. These F_1 isolates were then used as females in crosses with 4 West Virginia isolates, two of which were A group and two of the B group. The results are presented in TABLE III. It was necessary to make reciprocal crosses to determine the compatibility group of some of the isolates and to determine that they could act as males.

TABLE III shows that the 23 single ascospore cultures of cross *633*, dark B ♀ × *805*, light A ♂ segregated into four groups as follows: 14 dark B ♀, 2 dark A ♀, 6 light B ♂, and 3 light A ♂. These results show that the character unisexual male is inherited genitically and that isolate *805* is genitically unable to act as female. The factor for compatibility is not linked with sex or with color of mycelium. In the particular cross above dark mycelium segregated with the bisexual character and light mycelium with the unisexual male character.

The above cross was repeated and of the 57 single-ascospore cultures obtained 27 were dark and 30 were light. In crosses using these F_1 isolates as females and *613* and *638* as males, 24 of the dark isolates produced perithecia. Three dark cultures and all of the light cultures failed to act as females. This is further evi-

TABLE III

RESULTS OF CROSSING SINGLE ASCOSPORE CULTURES OF THE CROSS (*633* ♀ × *805* ♂) AS ♀ WITH FOUR WEST VIRGINIA CULTURES AS ♂

♀ \ ♂	B *611*	B *613*	A *620*	A *638*	Compatibility group	Color	Sex
7	O	O	P	P	B	dark	bisexual
8	O	O	P	P	B	dark	bisexual
9	O	O	P	P	B	dark	bisexual
11	O	O	P	P	B	dark	bisexual
14	O	O	P	P	B	dark	bisexual
15	O	O	P	P	B	dark	bisexual
16	O	O	P	P	B	dark	bisexual
17	O	O	P	P	B	dark	bisexual
19	O	O	P	P	B	dark	bisexual
20	O	O	P	P	B	dark	bisexual
21	O	O	P	P	B	dark	bisexual
23	O	O	P	P	B	dark	bisexual
5	P	P	O	O	A	dark	bisexual
18	P	P	O	O	A	dark	bisexual
2	O	O	O	O	B*	light	male
4	O	O	O	O	B	light	male
6	O	O	O	O	B	light	male
10	O	O	O	O	B	light	male
22	O	O	O	O	B	light	male
24	O	O	O	O	B	light	male
12	O	O	O	O	A	light	male
13	O	O	C	O	A	light	male
15	O	O	O	O	A	light	male

* Compatibility of male cultures was determined by reciprocal crosses.

dence that there may be genetic linkage between color and sex. Loss of femaleness is apparently usually accompanied by loss of dark color. However, the inheritance of color is not fully understood and may be complex.

Since it is possible that the medium may affect the fertility of a cross, several of the unisexual male F_1 isolates were tested in duplicate crosses on chestnut meal agar, a medium used by some workers for the production of perithecia. The results on this medium did not differ from those on the standard maltose-Casamino Acids agar.

DISCUSSION

While the finding of unisexual male cultures of *C. quercina* is not surprising, it lends evidence to the idea held by many that this

fungus is quite variable and changeable. It is not known whether these light colored unisexual male mutants occur in nature, but there is evidence that this degenerate change occurs rather frequently in culture.

Several isolates, some of which have been in culture for less than one year, have failed to act as female in a limited number of crosses. Although genetic proof is available at present for only one isolate, it seems probable that a number of these isolates are true unisexual males. Their frequency might explain, at least in part, the difficulty some workers have experienced in obtaining perithecia in culture.

The medium and the method of growing and spermatizing the cultures described above were developed specifically for the production of perithecia. The method is similar to that used by Hepting *et al.* (**5**). They are now believed to be sufficiently dependable that only two or three repetitions are necessary to establish that a certain cross is sterile.

The similarity between *C. quercina* and *Hypomyces solani* f. *cucurbitae* is again apparent in the existence of unisexual males, reported for the latter fungus by Hansen and Snyder (**2**). When cytogenetic studies of *C. quercina* are made they should be compared with the findings of Hirsch (**6**) in *H. solani* f. *cucurbitae* to determine whether the loss of femaleness can be explained on the same genetic basis in both fungi.

SUMMARY

In crosses between isolates from Minnesota and West Virginia, culture *805* failed to act as female in repeated trials. When this culture having light mycelium was crossed with a bisexual culture with dark mycelium, the 23 F_1 single ascospore cultures segregated into light male and dark bisexuals. The compatibility factors separated independently of sex or color of mycelium. Other isolates have shown this apparent inability to act as female. It is not yet known whether unisexual male mutants occur in nature.

DEPARTMENT OF PLANT PATHOLOGY
AND BACTERIOLOGY,
WEST VIRGINIA UNIVERSITY,
MORGANTOWN, WEST VIRGINIA

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ADDITIONAL OBSERVATIONS ON THE CHROMOSOMAL STRUCTURE OF THE VEGETATIVE NUCLEUS OF BLASTOMYCES DERMATITIDIS [1]

EDWARD D. DELAMATER

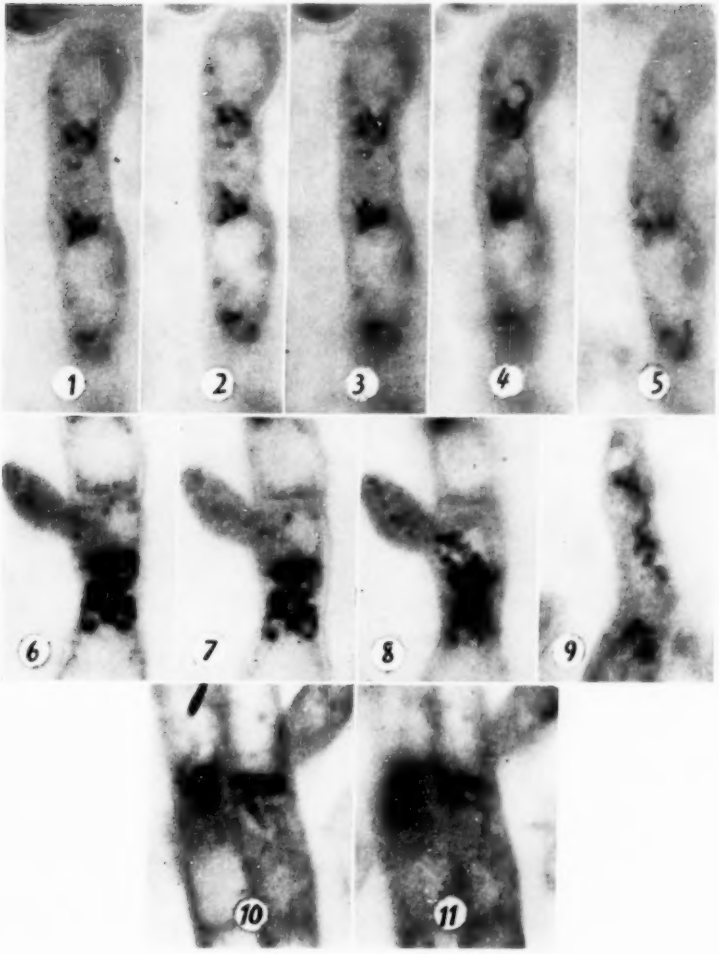
(WITH 11 FIGURES)

In a previous study of the nuclear cytology of *Blastomyces dermatitidis* (**1**) by means of new methods (**2**), the author described the nuclei and their mitotic activity in relative detail. The accompanying plate presents additional observations on the chromosomes of this organism.

The methods used are the same as those previously described. The cells of *B. dermatitidis* are multinucleate. FIGS. 1–5 show five successive focal planes through the same cell, and demonstrate two nuclei lying at either end of a cytoplasmic band which bisects the cell. The nuclei lie within the cytoplasm and adjacent to the vacuoles, but are distinct from the vacuoles. The chromosomes within the upper nucleus, which is in interphase, are especially well seen as long, delicate, beaded threads.

FIGS. 6–8 represent three focal planes through the same cell, showing a cluster of interphase nuclei, again within which the chromosomes appear as tangled, beaded threads. The dark granules in the nuclei in FIG. 6 represent chromosomes in cross section. In FIG. 9 in a dying cell are seen thread-like beaded chromosomes. The nuclear membrane has been disrupted. In FIGS. 10 and 11 are shown two optical levels through a prophase nucleus in which four contracting and beaded chromosomes were made out in the original preparation.

[1] Department of Dermatology and Syphilology, and Department of Microbiology, School of Medicine, University of Pennsylvania.



Figs. 1–11.

CONCLUSION

The observations presented add to those previously documented concerning the nature of the chromosomes and their number in the multinucleate cells of *B. dermatitidis*. These observations are presented because it is felt that additional evidence concerning chro-

mosomal structure and activity in the bacteria and fungi will be useful.

UNIVERSITY OF PENNSYLVANIA,
PHILADELPHIA 4, PENNSYLVANIA

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NOTES ON SOME MYCOLOGICAL METHODS

M. J. Thirumalachar and M. J. Narasimhan

(with 1 figure)

During the course of mycological and plant pathological studies the authors have been employing for the last several years some research techniques which have been found to be very useful and warrant recording.

1. A water mounting medium for making permanent microscopic preparations

The need for a medium which would render the slides permanent by direct mounting from water or lactophenol has been keenly felt by all students of fungi. The benefits of preserving permanently hundreds of interesting observations made under the microscope have been lost in most cases because the mounts are temporary and can be preserved only for a few weeks or months. Various mounting methods are employed which make the microscopic preparations fairly permanent, but each of them has certain disadvantages of its own. Glycerine jelly, for instance, has been widely used for mounting by students of fungi. While these mounts are fairly permanent, the material becomes opaque and does not have the clarity of objects mounted either in lactophenol or xylol-canada balsam. The double cover-glass method of mounting devised by Diehl (**1929**) and further modified by Chupp (**1940**) is really advantageous in mounting filamentous algae and fungi in the absence of any other mounting medium of better quality. Apart from the expensiveness of using two cover-glasses for sandwiching the material, the retention of even a little trace of water in the medium after heating makes the mounts milky-white and opaque due to admixture with xylol used along with canada balsam. Secondly, having made a chance observation of an in-

teresting stage of a fungus under the microscope, it is difficult to transfer the material *en bloc* without mutilation to another cover-glass for making the permanent mount according to the double cover-glass method.

The use of lactophenol as a medium for mounting and staining the materials of fungi has been widely employed. Dade and Waller (**1949**) used a sealing wax mixture consisting of equal parts of refined beeswax and gum dammar for sealing the margins of the cover-glasses of lactophenol mounts and thus rendering them semi-permanent. Thirumalachar and Pavgi (**1950**) found that the use of canada balsam of thick consistency as an additional coating over the beeswax-gum dammar sealing medium made the mounts more permanent even under the conditions prevailing in the tropics. However, in all these cases the purpose in sealing the margin of the cover-glasses is to create an effective barrier against the outflow of the lactophenol which often happens even with the slightest break of contact between the slide and the sealing medium. In the present studies the authors have been employing a water mounting medium which renders the mount permanent and hard, like canada balsam, and at the same time retains the clarity of the objects. The medium has the following composition:

Gum arabic	20 gm
Chloral hydrate	10 gm
Dextrose	10 gm
Glycerine	3 ml
Distilled water	30 ml

Powdered gum arabic is first placed in distilled water until it becomes a uniform gel. Chloral hydrate and dextrose are added to this and slightly warmed until a uniform viscid solution is formed. Glycerine is added to this solution and well mixed by stirring.

For making permanent mounts the material on the slide may be sealed with a cover glass using the water mounting medium. Where staining of the material is required, the objects are stained with cotton blue or acid fuchsin in lactophenol and slightly warmed until proper differentiation is obtained. After this, any excess of lactophenol is drained off and the material mounted in the water

mounting medium described above. The slides are then placed in an incubator or on hot plate at 40 to 45° C for 48 hours. When the medium has dried up and become sufficiently hard, the margin of the cover glass is streaked with a solution of medium consistency of canada balsam in chloroform and the slides are left on the hot plate for a few hours. This additional coating of canada balsam on the dried water mounting medium along the margin of the cover glass, prevents the possible re-absorption of moisture and consequent softening which sometimes happens under excessively humid weather conditions.

Depositing of the type specimens in important herbaria of species of fungi described as new to science is considered by mycologists as important. While these serve the purpose of being available for future comparative studies, in many fungi, such as species of *Sclerospora*, the herbarium material is without value for studying the conidial stages. Even so, in genera of fungi such as *Phyllosticta*, *Cercospora* and several others, the spores disappear after a few years of storing in the herbarium. It would be in the interest of mycological science to insist on the deposit of at least three slides made from the type material to be included in the same packet with the type specimen. This would enable the person making comparative studies at a later date to obtain the picture as seen by the author of the species. Considering the ease with which permanent mounts could be made by using the water mounting medium described above, we feel confident that the depositing of the slides with the herbarium material of the type specimen would not be a difficult task.

2. AN IMPROVED METHOD FOR GERMINATING AND STAINING THE SPORES OF FUNGI

For germinating and staining spores of various fungi such as the teliospores of rusts, chlamydospores of smuts etc. one of us (Thirumalachar, **1940**) described a procedure for fixing the spores to the slides. The spore material to be germinated was fixed to the slide by alternate wetting and drying of the spore mounts. The spore material thus fixed to the slide was inverted over a water surface supported by racks, and wet paper toweling was

placed on the back of the slide to help condense films of water on the inner side. Use of small card-board boxes which were rendered water-proof by dipping in molten paraffin was recommended by Thirumalachar and Pavgi (**1950**) as improvised moist chambers for incubating the slides. Further experience with this germination technique showed that when the germinated spores were carried through staining solutions, grades of alcohol etc. in the process of staining, several of the spores got dislodged from the slides and floated away, resulting in the loss of valuable stages in the germination. The writers have been investigating for the last several years the possibility of finding an adhesive which could be used to fix the spores to the slides prior to placing them for germination. Such an adhesive should meet the following requirements: (a) the adhesive should not inhibit or interfere with the spore germination; (b) it should not be dissolved in water and thus loosen the spore mass whch has been fixed to the slide; (c) it should not incite the growth of bacteria and other contaminants during incubation in moist chamber; (d) it should not show avidity for the stains used for staining the germinating spores. A large number of gums from different plant sources: resins, gum dammars, albumen, etc. were tried without success. Mucilages of various plants were also tried. Of the several that were tried, the mucilage from the stems and flowering stalks of *Tradescantia* sp. (*T. virginiana* L.?), a plant grown in almost every garden for its bright colored foliage, was found most useful. The writers have used this for fixing the spores to the slides with excellent results and feel confident that this germination technique would afford a means to get at critical cytological stages of germinating spores which hitherto has been found difficult on account of lack of a proper method of affixing the germinating spores to the slides for staining. The outline of the procedure for mounting, germinating and staining of the spores is as follows:

The spore material to be germinated is mounted in a droplet of water on a slide. Flowering stalks or young shoots of *Tradescantia* are freshly cut, and by pressing the cut end with the fingers, a tiny drop of the viscid mucilage is squeezed out which is then mixed with the spore material on the slide. If due to some

stage of maturity of the *Tradescantia* plant the mucilage is watery or of thin consistency, such shoots should not be used. With the aid of a needle or scalpel a uniform smear is made which is then air dried. The large number of raphides which are present in the mucilage disappear completely during the process of fixing, mordanting and staining. By alternately moistening and drying the slides, the material gets firmly fixed to the slides. Steeping the slides for a period of 10 to 20 minutes in a solution of potassium permanganate (0.01% or two tiny crystals for a glass of water) has been found to reduce contamination and stimulate spore germination in most of the cases studied. The slide, with the spore material thus fixed, is inverted over a water surface supported on racks and with wet paper toweling on the back side to help in condensing films of moisture by the methods already described (Thirumalachar, **1940**; Thirumalachar & Dickson, **1947**).

When sufficient numbers of spores have germinated, as determined by examination under the microscope, the material is fixed by the following procedure: excess of water surrounding the spore mount is wiped off and a small drop of Allen's modification of Bouin's fluid (with 1% urea) is added and spread over the spore mount which is then allowed to air dry. Immediately after the fixing solution dries, the slides are placed for 5 minutes in water and then transferred to Allen's modified Bouin's fluid in a coplin jar for 2 to 6 hours, depending upon the nature of the material. The slides after fixation are washed, stained, dehydrated in grades of alcohol and mounted in xylol-canada balsam by following the usual procedure adopted for paraffin sections.

The drying up of the slide after the addition of a droplet of Allen's modified Bouin's solution does not cause any shrinkage or distortion of the contents of the delicate germ tube. Actually, on the addition of the fixing fluid (containing picric acid and acetic acid), the mucilage swells, and on drying it prevents shrinkage of the delicate germ tubes. Immediate transfer of the slides to water after the few minutes drying completely restores to normal any possible distortion that might have taken place. The drying up of the slide has a special advantage in cases such as teliospore germinations. In these, usually, the promycelia bearing the sporidia

grow away from the water surface and only the bodies of the teliospores are fixed to the slide, while the promycelium and sporidia remain free-floating. By this drying process even these get fixed to the slide and there is no loss of sporidia etc. from the spore mount.

3. A METHOD FOR PRESERVING AND TRANSPORTING PLANT MATERIALS IN FRESH CONDITIONS

The need for getting diseased plant material in a fresh condition is very essential when isolation and cultural studies of the disease-inciting organism are planned. In many cases the period between

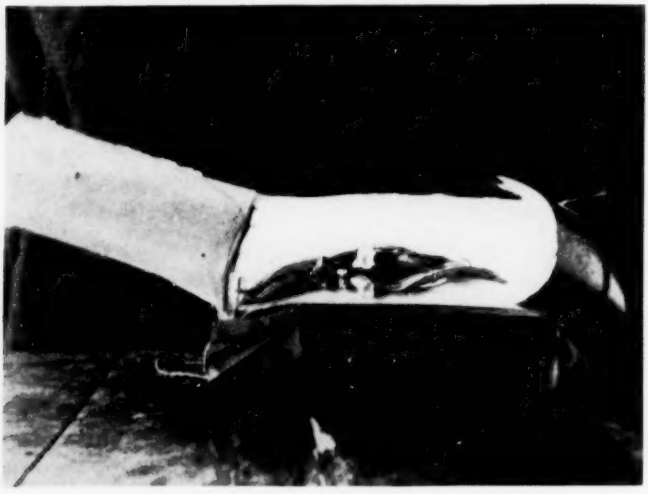

FIG. 1. Photograph of a specimen packet made from the banana leaf sheath, opened to show the specimens and the method of folding of the banana leaf sheath.

the time of collection of the diseased material and the time of microscopic examination may be more than a week (as for instance in the case of materials collected during mycological forays). If the material could be kept in a fresh state for the period it would always permit a more critical study.

Several methods employed at present are as follows: wrapping the material in water-proof paper, celluloid cases, packing in moss

and peat etc. These help to retain the moisture and viability of the spores but do not preserve the fresh condition of the host with the parasite.

In the course of collection and studies of fungi in several parts of India, often in sweltering heat, the writers have found the use of leaf sheath of banana extremely useful. The aerial part of the plant is a pseudostem covered with the sheathing leaf bases. These sheathing leaf bases are green outside, succulent and boat-shaped. By folding on either side, a hollow chamber is formed in which the specimens can be placed (FIG. 1). By firmly tying with a string, the margins of the sheath come together and form a close moist chamber. Fresh plant materials when collected and placed inside this have remained in a fresh state for over 12 days in spite of the rough conditions during transit by postal or railway parcel. Material when taken out at the end of this long period appears as fresh as if collected a few minutes before. To test the efficacy of this method, diseased plants affected by bacterial plant pathogens were despatched by railway parcel. At the end of 10 days the material was found to be in a very fresh condition, enabling the isolation and cultural study of the pathogen. By employing this method, material may be despatched from Puerto Rico to Wisconsin or from the Gold Coast of England by parcel post and received at the destination in a fresh condition to enable critical studies.

4. BANANA LEAF SHEATH CHAMBER FOR INOCULATING EXCISED LEAVES OF PLANT PATHOGENS

The petri dish method of inoculation described by Clinton and McCormick (**1924**) for inoculating excised leaves with rust spores is used in several laboratories. This method enables one to carry out inoculation experiments in cases where potted plants of the host are not available and also ensures protection against contamination from air-borne spores. The longevity of the leaves under the conditions prevailing in the petri dishes varies from plant to plant, some of the thin-leaved plants being susceptible to early decay. In the present studies, it was noticed that in every case the banana leaf sheath supported the host leaves in a fresh condition for 5 to 6 days more than the petri dishes during the same period of incu-

bation. It was therefore found useful to use the banana leaf sheath chamber for incubating excised plant leaves and shoots after inoculating them with the required plant pathogen. This method has been found very useful in carrying out inoculation studies and obtaining critical data even in remote field stations where the usual laboratory facilities are not available.

MALLESWARAM, BANGALORE,
INDIA

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SAMUEL CHESTER DAMON II

WALTER H. SNELL

(WITH PORTRAIT)

Samuel Chester Damon II was born in Baltimore, Maryland, on November 18, 1923, the son of Samuel Reed and Jeannie Reid Damon. After completing his high-school education at Towson, Maryland, and Montgomery, Alabama, he entered Brown University in 1940 and remained until the fall of 1943, receiving his B.A. degree with honors in Botany, with the class of 1944.

After one day in the army, he received his discharge in order to allow him to enter the navy and he was commissioned as an Ensign early in 1944. His indoctrination was followed by training at the Bomb Disposal School in Washington and he served as bomb-disposal officer in the South Pacific. He completed his service on August 1, 1946, as Lieutenant (j.g.), with the following authorized decorations: American Area Campaign Medal; Asiatic-Pacific Area Campaign Medal (2 stars); European-African-Middle-Eastern Area Campaign Medal; Philippine Liberation Ribbon; World War Two Victory Medal.

As an undergraduate, Damon, like most young men of the period, was unsettled as to his professional future. He took his concentration in botany and bacteriology but could demonstrate no particular liking for anything in either field at that time. The best interest he could show in mycology was to honor the author of this article by auditing his lectures. During his war service, however, he developed an enthusiasm for medical mycology and returned to Brown in the fall of 1946 for graduate work in mycology. In this period, he took a short course in medical mycology with Dr. Norman F. Conant at Duke University. Even though this special field continued to be a mycological avocation with him, for his two graduate theses he investigated certain imperfect fungi. He was awarded the Ph.D. degree by the University of Iowa in 1951

SAMUEL CHESTER DAMON II

after three years of study under Dr. G. W. Martin. He began his professional work immediately as Taxonomic Mycologist in the Research and Developmental Laboratories of the Philadelphia Quartermaster Depot of the U. S. Army.

Damon was married to Miss Meffie Scales of Iowa City in April, 1949, and a son, Samuel Reed Damon III, was born to them on January 28, 1950.

Toward the end of his graduate work at Iowa, he underwent a minor gland operation which could have been only a minor ailment. It was not long, however, before the trouble proved to be more insidious—Hodgkin's disease. He soon realized that his time was limited and that his work was to end in the near future. Nevertheless, he faced the facts with indomitable courage and true bravery—at times almost with detachment, it seemed, as he followed in detail the progress of the disease—planning farther research and working doggedly each day to the limit of his waning strength, until the moment he was compelled to return to his family home in Kingston, R. I., for the end he clearly foresaw. In a little more than a year after the onset of his disease, he died, in Kingston, on August 8th, 1952.

This young man was endowed with a clear and keen mind, a high degree of intelligence, and an excellent critical sense. He not only had broad scientific interests and training but he had a wide acquaintance with literature and music, and his knowledge of history provided a discerning understanding of current events. He had a well developed bump of scientific curiosity. At times, apparently interested in nothing in particular and making no noticeable effort, he would suddenly turn up with the most unusual and recondite material from the strangest places, and all in such time that one would wonder not only when but where and how he obtained it. It never paid to enter an argument with him on any subject without being quite certain of one's facts. He was accused of working all night every night but libraries do close and the present writer happens to know the hours kept by this young man.

The lighter side of Sam Damon's character was most unusual and fascinating. He was a jovial companion, with a delicious sense of humor. His enjoyment was expressed variously with a

sly twinkle, a quiet and throaty chuckle or with head thrown back, a contagious guffaw of laughter from the depths. He loved to josh, even rather violently at times, with his best friends; he enjoyed to the utmost being ribbed as well as ribbing. He delighted in assuming deceptive attitudes and postures, giving wrong impressions to the uninitiated. He cultivated an air of extreme cynicism. He habitually portrayed himself as an apostle of laziness and extolled the virtues of laziness as a manner of genteel living. With pipe in mouth, feet on desk, lids half closed over keen eyes—a picture of complete relaxation—he appeared to be doing little but loafing leisurely and accomplishing nothing by the hour, but the wheels were always turning and the results produced were astounding, albeit in no spectacular manner.

To those of us who knew him well, it will be long before anyone can fill his place in our laboratory, because of his keenness behind a feigned stupidity, his seriousness disguised by a superficial frivolity, his accomplishments in spite of his pretended laziness and seemingly lackadaisical responses, the soundness and solidity of his character, and his deep loyalty to persons and institutions beneath an assumed cynical and even flippant attitude. It will be long before we have another student so enjoyable and gratifying personally, socially and academically, and with such obviously uncommon promise for mycology as Sam Damon.

His herbarium, mostly of Fungi Imperfecti, and including 9 types, has been presented to the New York Botanical Garden.

He was a member of the Society of the Sigma Xi, the Mycological Society of America, and American Association for the Advancement of Science, the Torrey Botanical Club, and the Scandinavian Society for Plant Physiology. The results of his scientific work were published or are projected as follows:—

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BROWN UNIVERSITY,
PROVIDENCE 12, RHODE ISLAND

NOTES AND BRIEF ARTICLES

A Simply Prepared Identification Medium for Candida albicans [1]

In routine laboratory procedure, *Candida albicans* is usually distinguished from the non-pathogenic species of the genus by the formation of large, thick-walled chlamydospores on corn meal agar. This characteristic suffices for routine identification, and usually renders unnecessary the more elaborate fermentation reactions and rabbit inoculation (Conant *et al.*, Man. Clin. Mycol. 1944).

The main purpose of corn meal agar is to provide a medium poor in nutrients which favors the production of chlamydospores. The preparation of this medium is, however, both laborious and time-consuming (Lewis & Hopper, Int. Med. Mycol. ed. 3. 1948).

The following identification medium is easily and quickly prepared and induces the formation of chlamydospores as satisfactorily as corn meal agar.

Required ingredients:

White rice	20 grams
Tap water	1000 ml
Agar	20 grams

Cover the rice with the water and bring to a boil. Allow to boil over a low flame for 45 minutes, keeping the vessel covered.

Then filter the liquid through gauze and allow it to cool. Discard the rice.

[1] From the Department of Dermatology and Syphilology of the New York University Post-Graduate Medical School (Dr. Marion B. Sulzberger, Chairman) and the Skin and Cancer Unit of the New York University Hospital.

After cooling, add tap water to the liquid to make 1000 ml. Add 20 grams of agar, and heat the mixture over a low flame or in a water bath until the agar is dissolved.

Sterilize the medium for 15 minutes at 17 pounds pressure and 120° C and pour into sterile Petri plates, or measure into test tubes before sterilizing, sterilize, and slant.

Chlamydospores were observed consistently and in abundance 17 to 24 hours after inoculation of this medium with *Candida albicans*. The morphology of both the pathogenic organism and the non-pathogenic Candidas agreed in every respect with that of the cultures usually obtained on corn meal agar.

With the addition of 2% dextrose C.P. this rice infusion medium may also be substituted for corn meal in the differentiation between *Trichophyton mentagrophytes* and *Trichophyton rubrum* (Bocobo & Benham, Mycologia **41**: 291. 1949).—CLAIRE L. TASCHDJIAN.

A NEW NAME FOR VELUTARIA RUFO-OLIVACEA

It is now apparent that no valid generic name is available for *Velutaria rufo-olivacea* (Alb. & Schw. ex Fr.) Fckl. (and its allies) so long as the present International Code of Botanical Nomenclature (Regnum Vegetabile **3**: 49, 50. 1952.) requires us to accept *V. grisco-vitellina* (Fckl.) Fckl. as the lectotype of the genus *Velutaria*. I earlier (Mycologia **45**: 296–301. 1953.) showed that this typification would be required, but stated that the generic name *Phacangella* (Sacc.) Sacc. & Sacc. (Syll. Fung. **18**: 128. 1906.) might be available for the *rufo-olivacea* group, provided the lectotype selected by Petrak (Sydowia **3**: 199. 1949.), *P. aceris*, should prove to be a synonym and also to be the first lectotype designated. However, I overlooked the fact that Massee (Brit. fungus-fl. **4**: 136. 1895.) had raised Saccardo's subgenus to generic rank eleven years earlier. He included only three species in the genus, and no type was designated, but of these only *P. ulicis* (Cooke) Massee was included in *Cenangella* subgen. *Phacangella* Sacc., and is thus the only species eligible as the type of the genus *Phacangella* (Sacc.) Massee. According to Nannfeldt

(Nova Acta Reg. Soc. Sci. Upsal. 4, **8**(2): 83. 1932.) this species is probably a *Dermatea* (*Dermea*) or a *Pezicula*.

Unless the rules are changed to take into account predominant usage, *"Velutaria" rufo-olivacea* is left without a valid generic name, or the name must be conserved. While a number of species have been described in this genus (sensu auct. non Saccardo), only one is at all certainly distinct. It is difficult to make a good case for the conservation of such a small (probably monotypic) genus, and I have abandoned hope of effecting such conservation. I propose, therefore, a similar generic name, **Velutarina** nom. nov., to replace *Velutaria* Fuckel, Symb. myc. 300. [1870.] *sensu* Rehm *in* Rabenh. Krypt.-Fl. **1**(3): 645. 1892 (non *emend.* Sacc. Bot. Centralbl. **18**: 219. 1884.; Syll. Fung. **8**: 488. 1889.). The type species of the genus is **Velutarina rufo-olivacea** (Alb. & Schw. ex Fr.) comb. nov. (basonym: *Peziza rufo-olivacea* Alb. & Schw. ex Fr. Syst. myc. **2**: 99. 1822.).

The generic name *Velutaria* Fuckel *emend.* Sacc. must be retained as a genus of the Hyaloscyphaceae, with its type species *V. griseo-vitellina* (Fckl.) Fckl. *Tapesina* Lambotte *emend.* Höhn., as used in my monograph of the Arachnopezizeae (Lloydia **14**: 129–180. 1951.), falls into synonymy.—RICHARD P. KORF.

DISPOSITION OF NOMINA GENERICA CONSERVANDA PROPOSITA FOR FUNGI

As a result of a second ballot on the original proposals concerning *Tricholoma* (cf. Mycologia **45**: 321), the following was approved by the Special Committee for Fungi, and should be added to the list of proposals adopted under paragraph V (p. 317):

Tricholoma (Fr.) Kummer, Führer Pilzk. 25. 1871.—T.: *Agaricus flavovirens* A. & S. ex Fr., Syst. Mycol. **1**: 41. 1821 (cf. *A. equestris* Fr., Elench. Fung. **1**: 6. 1828).	*Tricholoma* Benth. in DC., Prodr. **10**: 426. 1846.—T.: *T. clatinoides* Benth., l.c.

—DONALD P. ROGERS, Secretary, Special Committee for Fungi.

REVIEWS

Les Agaricales II. Russulacées, Hygrophoracées, Gomphidiacées, Paxillacées, Boletacées, by P. Konrad and A. Maublanc. (Encyclopedie Mycologique XX); P. Lechevalier, Paris. 202 pp., unbound, Price 2200 francs.

The second volume of this work follows rather closely the general organization of material adopted for the first volume. It treats the families mentioned in the title as opposed to the entire remainder of the Agaricales which were combined in the first volume as Agaricaceae. The Hygrophoraceae and Gomphidiaceae consist exclusively of their respective type genera; *Paxillus* and *Phylloporus* form the Paxillaceae, and the Boletaceae are subdivided in two subfamilies (Boletineae and Strobilomycetineae) and 10 genera. The authors have continued to follow a middle course between modern and conservative concepts, an attitude that may have its educational merits, for this work will undoubtedly have influence on the shaping of taxonomical thought of amateurs as well as beginners for which the work is mainly intended. The presentation of a "middle of the road" classification will tend to introduce the progress of systematics during the last thirty years in a gentle, easily digestible and prudently eclectic manner. The book gives a generally true picture of what the average of the mycologists of the foremost country in agaricology, France, is willing to accept at present. The high standard of French mycology, best illustrated by the series of publications in Lechevalier's Encyclopédie Mycologique which has produced such classics as Heim's *Inocybe* monograph and Kühner's *Mycena* studies, is maintained in volume XX of this collection here reviewed. The enumeration of the species is up to date and reasonably complete, and the short diagnoses in the Linnean spirit are useful for a fuller understanding of the relative position of each species. The American reader will find the work useful as a synopsis of the European agaric and bolete flora and will use it for this purpose together with Pilát's

recent "Klic" (Key); he will, however, perhaps more than the European reader, wonder about the scarcity of references to non-European literature on the subject, and the multitude of cases of disregard for the rules of nomenclature. Earlier specific epithets are often given as synonyms (*Boletus aeruginascens, Boletus floccosus, Boletus pseudoscaber, Pluteus atromarginatus* (Konrad) Sing.), and the quotation of the author is very frequently inexact. The consultation of American type studies would have avoided misinterpretation of such species as *Russula pectinatoides, R. ochroleucoides, R. turci, R. neglecta,* etc. These examples could easily be extended, yet it may be said that Konrad and Maublanc's work is rather less remarkable for these tendencies than that of other contemporaneous European writers. The minor shortcoming just mentioned cannot distract from the conclusion that Konrad & Maublanc's Agaricales should be part of every mycological library.—ROLF SINGER.

RUSSULA-MONOGRAPHIE (DIE PILZE MITTELEUROPAS, BAND III), by Julius Schäffer, Verl. Julius Klinkhardt, Bad Heilbrunn, Austria. 296 pp., 20 plates, price 7 pounds 10 sh.

The book consists of a text volume which is a reedited copy of the original Russula-Monographie (Annales Mycologici **31**: 305–516, 1933 and **32**: 141–243, 1934) and the plates partly published as vol. III of the Pilze Mitteleuropas and now added to the revised text. The work is, consequently, partly posthumous, and the revision consists merely in the editing of later published and unpublished notes left by Schäffer and inserted by A. Flury, Basel. The republication of the original monograph is in itself of questionable usefulness, but may be justified insofar as it was apparently considered necessary to add some finished text to the plates. The additions, however, have been edited very poorly and the new text contains many more printing errors than the original. Naturally, many papers have been published on *Russula* between 1933 and 1952, yet they were neither entirely ignored, nor were they fully taken into consideration. Thus, *Russula ferreri* Sing. was introduced although Schäffer never knew it and no plate is available; on the other hand, there is no trace of important new species like

R. chamaeleon Sing., or the results of new type studies as on *R. gracilis* Burl. (*R. gracillima* J. Schäffer), *R. blackfordiae* Peck (*R. versicolor* J. Schäffer), *R. pulchella* Borszcz. (*R. palustris* Peck, *R. exalbicans* (Secr.) sensu Melzer & Zvára, *R. depallens* Fr., sensu J. Schäffer), *R. mariae* Peck, etc. Even the editing of the unpublished notes has been done carelessly; one of the most obnoxious cases of this sort is the verbatim reprinting of the description of *R. olivascens* (no. 38) and the inserting of an added species, *R. chamaeleontina* (no. 41) whereby the text reveals that J. Schäffer's real intention was to combine the two species under the name *R. chamaeleontina*, which is obvious because of the fact that *R. olivascens* is indicated as a synonym of *R. chamaeleontina*. The reviewer as well as the majority of European specialists does not agree with this opinion, as *R. chamaeleontina* is obviously an outstanding example of a nomen dubium and a nomen ambiguum, and the identity of R. no. 38 and no. 41 is improbable, yet the point is that the editor did not understand Schäffer's position and the result is an inconsistency which goes through the entire book. While it is still argued that *R. raoultii* Quél. is "unterbestimmt" (not completely known)—p. 218—or systematically valueless (p. 216), the species must have had some meaning to the author since a good colored picture is given in the second volume. The additions to the keys (pp. 262–288) are equally unfortunate. *R. cremeoavellanea* is wrongly inserted among the species with the context turning blackish gray and is entirely absent in the "Synoptische Tabellen." There is no use in emphasizing the points on taxonomy or nomenclature in which this reviewer and Schäffer disagree; they have been discussed in various publications, and should not be rediscussed on this occasion. In spite of their shortcomings, Schäffer's views have found a considerable degree of acceptance in European publications, and this tendency will perhaps grow as long as Schäffer's monograph is the latest and most easily available contribution to the knowledge of the genus. However, it must be stated that the present edition of the monograph does not contribute much to the general knowledge of the Russulae, and rather confuses some of the issues. On the other hand, the illustrations are very valuable and it is indeed helpful, especially for

the beginner, to have another atlas added to those already existing in Europe, such as Melzer's excellent but smaller "Atlas Holubinek" (Praha 1945). In quality of design, color, and reproduction, Schäffer's figures are undoubtedly superior to most other publications containing European Russulae, and the fact that these plates actually represent the majority of Europe's Russula flora contributes greatly to their value.—R. SINGER.

SOIL MICROBIOLOGY, by Selman A. Waksman. New York: John Wiley and Sons, Inc. 356 pages (illus.), 1952. Price $6.00.

This book by a noted authority in the field of microbiology was designed as a textbook. It includes considerable material, including tables and illustrations, found in two previous books, Principles of Soil Microbiology (1927), and The Soil and the Microbe (1931). As indicated in the preface the aims of this book were "to survey the nature and abundance of microorganisms in the soil, to review the important role that they play in soil processes and, as far as possible, to show the relation between them and soil fertility."

Although reference to and discussion of many investigations conducted in various parts of the world are presented, much of the material in the book naturally had its origin in the extensive researches of Doctor Waksman and his associates. It is not claimed by the author that all phases of the subject of the soil microflora are covered in a comprehensive manner. The systematic mycologist may, in fact, find a paucity of information on his favorite subject.—O. H. SEARS.

MANUSCRIPT

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